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WILLIAM DOW OSTROFSKY

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AMERICAN BEECH

*University of New Hampshire*

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EXOPHYLACTIC AND NECROPHYLACTIC PERIDERM  
DEVELOPMENT IN AMERICAN BEECH

BY

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B.S., University of New Hampshire, 1973  
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DISSERTATION

Submitted to the University of New Hampshire  
in Partial Fulfillment of  
the Requirements for the Degree of

Doctor of Philosophy  
in  
Botany

December, 1982



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THIS DISSERTATION IS DEDICATED TO  
MY WIFE

Andrea Ostrofsky

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## ABSTRACT

### EXOPHYLACTIC AND NECROPHYLACTIC PERIDERM DEVELOPMENT IN AMERICAN BEECH

by

WILLIAM DOW OSTROFSKY

University of New Hampshire, December, 1982

American beech (Fagus grandifolia Ehrh.) in forest stands of North America are threatened by a destructive disease commonly known as the beech bark disease. Investigations on bark structure and on response of bark tissues to mechanical injury and pathogen invasion would increase our understanding of the etiology of this disease. The objectives of this investigation were to provide descriptions of the development and anatomy of bark of American beech, with emphasis on bark periderms. Investigations were made of the ontogeny of the first exophylactic periderm, and on characteristics and development of the necrophylactic periderms resulting from the beech bark disease and from experimentally inflicted wounds.

The phellogen of the first exophylactic periderm in current year shoots was initiated within one week after budbreak in late April. This phellogen developed from the cortical cell layer immediately below the epidermis.

A phellem of five or six cell layers in thickness and a phelloderm of up to two cell layers in thickness were present by the end of the first growing season. First year periderm development was complete ten weeks after budbreak.

Nectria coccinea var. faginata was capable of inducing cankers on beech stems when applied to wounds made by the removal of only the first exophylactic periderm. Wounds inflicted and inoculated in the fall resulted in more cankers than those inflicted and inoculated in any other season. All cankers became delimited by a necrophylactic periderm within two years after injury.

When beech trees susceptible or apparently resistant to the beech bark disease were wounded and inoculated during the fall, initiation of the necrophylactic periderm did not occur until at least 30 weeks later. No differences were noted between susceptible and apparently resistant trees in rate of necrophylactic periderm development. Amounts of total bark phenolics during the development of delimited cankers was also investigated.

Anatomical evidence is presented which supports an hypothesis that the necrophylactic periderm which delimits naturally and experimentally induced cankers is generated from recent derivatives of the vascular cambium, as well as from living cells of the bark tissues present at the time of wounding or infection.

EXOPHYLACTIC AND NECROPHYLACTIC PERIDERM  
DEVELOPMENT IN AMERICAN BEECH

INTRODUCTION

American beech (Fagus grandifolia Ehrh.) is a primary constituent of the eastern hardwood forests of the United States. The only species of Fagus native to this country, it occurs in forest stands occupying approximately 6.1 million hectares, from northern Michigan south to eastern Texas, and eastward to the Atlantic Ocean. Beech is a principal component of four cover types (89), and as such has major ecological significance throughout its wide range. It is one of the most shade-tolerant hardwood tree species and, along with sugar maple (Acer saccharum Marsh.), commonly dominates the understories of most forest stands in which it occurs (90).

Beech is monecious, and trees begin to produce abundant seed when they reach approximately 40 years of age (91). The species also has the ability to form sprouts from stumps and roots (96). Sprouting from roots is believed to be a major method of reproduction, especially in disturbed, exposed, or climatically harsh sites (38).

Individual beech may attain a height of over 20 m and a diameter of over 50 cm at maturity. Stems of beech which have developed in relatively well stocked stands prune well naturally and exhibit good bole form (89). The bark, light

grey and smooth throughout the life of the tree, is one of the most striking characteristics of beech.

Beech in forest stands of North America are threatened by a destructive disease commonly known as the beech bark disease. The disease is the result of the combined activity of two primary causal agents, a scale insect and a fungus (29). The beech scale, Cryptococcus fagisuga Lind. feeds on living cells in the bark tissue, thereby predisposing the bark to subsequent infection by the fungus Nectria coccinea var. faginata Loh., Wats. & Ayers. Other species of Nectria may also be involved (26). Infection of the bark by this fungus results in the formation of cankers. When cankers are large and diffuse, or when many small cankers coalesce around the stem, tree mortality occurs.

The beech scale is thought to have been introduced to North America in Halifax, Nova Scotia in 1890, and the first outbreak of the beech bark disease was recorded in 1920 (29). Since that time, the disease has spread from Nova Scotia southwestward into the New England states of the United States, and is now found throughout New York and Pennsylvania. A recent report indicates that a large infestation of C. fagisuga now occurs in the Monongahela National Forest in West Virginia (D. R. Houston, personal communication).

The beech bark disease has also been known to occur throughout Europe since the mid-1800's (45). The principal fungus involved in the beech bark disease in Europe,

however, is Nectria coccinea (Pers. ex Fr.) Fries and the principal suspect is European beech, Fagus sylvatica L.

Recent investigations of the beech bark disease have centered upon the biology of the two primary causal agents, the beech scale (44, 92), and the Nectria species (25, 35, 54). Few researchers have investigated the role of the host in this disease complex, and their studies have been concerned with European beech (18, 33).

Investigations on the structure of American beech bark and the response of bark to pathogen invasion would provide the basis for a better understanding of beech bark disease etiology and epidemiology. More practically, a thorough understanding of the host in this host:parasite system may lead to better control recommendations or to studies in which more economically or ecologically acceptable control alternatives could be found.

An increased interest in studies on bark response to pathogen invasion has occurred during the last decade. This interest is due mostly to the critical and elucidating work of Mullick (61). Mullick has provided new insights regarding the role of anatomical and chemical changes of bark tissues with regard to normal plant growth and development as well as to mechanical and pathogen-induced injuries. His work has provided a new framework within which more critical questions may be asked of host:parasite systems involving woody plants.

The objectives of this dissertation are to provide

descriptions of the development and anatomy of bark tissues of American beech with emphasis on bark periderms, and to examine naturally and experimentally induced anatomical changes in bark tissues with respect to the beech bark disease. These objectives were met by conducting experiments which included:

- 1) An investigation of periderm ontogeny in current year shoots of American beech.
- 2) A description of the types of mature beech bark.
- 3) An investigation of the development of necrophylactic periderm in current year shoots of American beech.
- 4) An investigation of the relationship of exophylactic periderm to canker formation by Nectria coccinea var. faginata.
- 5) An investigation of necrophylactic periderms associated with various naturally induced cankers.
- 6) A comparison of necrophylactic periderm development in American beech trees susceptible to the beech bark disease with that in trees apparently resistant to the disease.
- 7) An investigation of the role of total extractable phenols of American beech bark with respect to bark infection by Nectria coccinea var. faginata.

## CHAPTER I

### LITERATURE REVIEW

#### Phellogen Initiation

Comparative investigations of woody plant periderms were made as early as 1845, and by 1900 a substantial amount of information on site of phellogen initiation, general periderm morphology, and the relationship of periderm development to bark configuration had been documented (83). These early studies were done in Europe and dealt primarily with European plant taxa. The relatively few studies dealing specifically with periderms of North American species are much more recent (12, 59, 68). Most information regarding periderms of woody plant species has been obtained from more general studies of bark (8, 21, 70) or phloem (31, 98).

Phellogen initiation in the development of first periderm, or exophylactic periderm sensu Mullick and Jensen (63), has been documented for a large number of taxa (27, 28). Duliott (28) cites Moeller as grouping taxa into four basic categories, based on site of phellogen initiation:

- i) Initiation occurring in the epidermal cell layer, as in species of Salix, Ilex, and most species of Cornus.
- ii) Initiation occurring in the cell layer immediately below the epidermis, as in species of Populus,



Platanus, and Acer.

- iii) Initiation occurring deep within the primary cortex but exterior to the vascular tissue, as in species of Larix, Robinia, and Cercis.
- iv) Initiation occurring within the region of the primary vascular bundle, as in species of Taxus, Vitis, and Podocarpus.

Phellogen initiation in European beech, described in 1882 by Moeller and later confirmed by Duliott (28), was found to occur in the cortical cell layer immediately below the epidermis. Few studies on phellogen initiation were reported during the 70 years following Duliott's comprehensive work. However, a renewed interest in periderms during the last 25 years has become evident, perhaps due to an interest in the physiological basis of periderm development.

Recent investigations have described the site of phellogen initiation for Ceratonia siliqua L. (1), Robinia pseudoacacia L. (2, 12, 94), Acacia raddiana Savi (3), Citrus limon (L.) Burm. (71), Populus tremuloides Michx. (68), Fraxinus pennsylvanica Marsh., Ailanthus altissima (Mill.) Swingle, and Pinus resinosa Ait. (12), Pseudotsuga menziesii (Mirb.) Franco. (81), and Eucalyptus camaldulensis Dehnh. (53). Chiang (22) has reported on periderm initiation in an additional 14 tree, 8 woody vine, and 19 herbaceous vine and shrub species. Of particular interest in Chiang's study was the discovery that the site of

phellogen initiation in Melaleuca leucadendra (L.) L. does not take place within one or two cell layers around the stem cylinder, but develops in an undulating band from the epidermis to the tenth cell layer of the cortex. This is apparently a unique way for phellogen initiation to occur.

#### Periderm Ontogeny

Although substantial information exists regarding the site of phellogen initiation, the time of initiation and seasonal activity of the phellogen, the rate of periderm development, and the influence of environmental factors on development have been described for only a few taxa. Most of these reports have appeared in the last two decades.

According to Srivastava (83), in 1860 Sanio described three developmental patterns of first periderms: centripetal, centrifugal, and reciprocal. The type of development characteristic of a species was determined by which of the two daughter cells resulting from cell division of the phellogen remained meristematic. Since most mature periderms have both phellem and phelloderm, the classification is most useful in describing periderm development only during its earliest phases.

Sanio has been credited by Srivastava (83) for the observation that periderm production was initiated in June for most species in the North Temperate region. DeBary (27) has made the generalization that in plants forming a periderm during the first year of growth, the periderm appears about the same time as differentiation of the

primary tissues begins. Srivastava (83) indicates that the phellogen arises uniformly around the circumference of the internode, and that an exception to this occurs in plants with a superficial periderm such as Citrus limon and certain Eucalyptus species. Phellogen initiation in these species, and also in Ceratonia siliqua (1), appears to arise in localized areas often at sites where lenticels will form. Borger and Kozlowski (12) reported that phellogen rapidly developed circumferentially in hypocotyls of Fraxinus pennsylvanica and Robinia pseudoacacia. However, in Ailanthus altissima four to five cell layers were present at regions where phellogen initiation had occurred before the phellogen had developed completely around the stem.

Rate of periderm development during the first season of growth varies between species, and is likely to vary even within a species if the taxon occurs over a wide geographic range. Esau (32) indicates that between two and twenty cell layers of phellem per radial file are produced during one year, depending on the species. A detailed report of periderm growth has been provided by Borger and Kozlowski (12), who compared rates of early growth in Pinus resinosa with that in Fraxinus pennsylvanica. Fifty-six days after cotyledon emergence, P. resinosa seedlings had developed three to four layers of periderm, while F. pennsylvanica had developed as many layers only 16 days after cotyledon emergence. Both species were grown under specific and identical conditions of moisture, temperature, light

intensity, and photoperiod.

The influence of environmental factors has been shown to be critical in determining time of phellogen initiation and rate of periderm development for several tree species. Arzee et al. (2) reported that when seedlings of Robinia pseudoacacia were exposed to relative conditions of long day length and high temperature, phellogen initiation occurred in lower internodes sooner than in those of trees maintained under conditions of either short day:high temperature, short day:low temperature, or long day:low temperature. The stimulatory effect of increased day length was also reported for Fraxinus pennsylvanica, Robinia pseudoacacia, and Ailanthus altissima by other investigators (10). Seedlings of these species, when germinated and grown in the dark for up to 56 days, failed to develop any periderm.

Light intensity has been reported to affect periderm formation differently, depending on the species. Phellogen initiation in Eucalyptus camaldulensis was reported not to be influenced when young stems were covered with opaque tape (53). Conversely, periderm initiation and rate of development in Pinus resinosa, Fraxinus pennsylvanica, and Robinia pseudoacacia was found to be positively correlated with increasing light intensity over the range of 70 to 1200 foot-candles of light (14).

The effect of temperature on phellogen initiation and development has been investigated for Fraxinus pennsylvanica, Robinia pseudoacacia, and Ailanthus altissima (15).

Periderm initiation in germlings of the latter two species was found to occur sooner, and periderm development occurred more rapidly with temperature increases from 10 to 25 C. Periderm development was retarded in these two species when grown at temperatures above 25 C. Periderm of Fraxinus pennsylvanica germlings increased in number of cell layers with temperature increases from 10 to 30 C.

In another investigation, Borger and Kozlowski (9) reported that periderm development of Fraxinus pennsylvanica was totally dependent on the presence of leaves, and suggested that leaves exported some substances stimulatory to periderm development. Fraxinus pennsylvanica seedlings grown under conditions of water stress severe enough to inhibit leaf formation also failed to develop a periderm (13). If water stress was not severe enough to inhibit leaf formation, then periderm developed, but less so than those of germlings provided with ample water.

The seasonal activity of the phellogen has been critically examined for only two species. Using a radioactive labelling technique, Arzee et al. (3) have provided evidence that the phellogen in Acacia raddiana twigs of trees grown in the Mediterranean region is active only during May, August, late October, and November. A similar technique was used by Waisel et al. (95) to measure phellogen activity in Robinia pseudoacacia. The phellogen of this species was determined to be active from late March through April, and from late June through August.

Srivastava (83) has suggested, however, that phellogen activity for most species is correlated more closely with the phenological events of shoot elongation and cambial activity.

Reviews by Borger (8), Srivastava (83), and Esau (31) have summarized a great deal of general information regarding rhytidome development and bark structure of mature woody plants. These authors indicate that it is a matter of general acceptance that Fagus sylvatica and other species of Fagus retain a superficial periderm throughout the life of the plant. Aspects of general bark structure of Fagus sylvatica have been provided by Holdheide as related by Whitmore (97), and Braun (18). Glase and Granet (34) have provided documentation regarding the photosynthetic activity of the bark of Fagus grandifolia.

#### Role of Periderms as Barriers to Pathogen Ingress

The bark of woody plants serves several functions including thermal insulation and prevention of dessication of the plant body, conduction of food reserves, photosynthesis, and protection of the vascular cambium from mechanical injury and pathogen invasion (8, 23, 32). A primary role of the strategically located phellogen is to maintain the protective layers of phellem by the generation of new cell layers in the event of their rupture or removal.

The phellogen is the first living tissue encountered by pathogens invading secondary plant parts, and it is often subject to physical rupture due to insect attack,

severe weather conditions, and other physical stresses. Due to the relative vulnerability of the phellogen to injury, plants also possess a mechanism whereby the generation of a new phellogen can occur. Wound periderms may be defined as those periderms which arise or are induced to develop due to the mechanical rupture or death of the existing phellogen.

The relationship of wound periderms to the development of diseases in woody plants has been investigated in a number of host:parasite systems. A few reports on wound periderm formation in response to invasion by bacteria (75) and parasitic seed plants (47, 85) have been provided. However, most reports on this subject deal with diseases caused by fungi.

Keefer (49) described cork layers which formed in bark tissues of Castanea dentata Borkh. infected with Endothia parasitica (Murr.) P.J. & H.W. And., the primary causal agent of the chestnut blight. Bramble (17) later made a more complete investigation of chestnut bark anatomy, and described the wound periderm anatomy associated with the disease. He suggested that wound periderm was at least a partial barrier to infection, since seedlings of C. dentata rapidly killed by the fungus had not developed wound periderm, while trees exhibiting partial resistance had developed a wound periderm. Bramble (17) also noted that the wound periderm consisted of up to ten cell layers of thin-walled phellem, four to six cell layers of thick-walled phellem, and three to four cell layers of phelloderm. This

pattern was quite different from the sequent periderms which normally develop in C. dentata after trees reach the age of approximately twelve years. The normal sequent periderms were reported to contain one or two cell layers of thin-walled phellem, six to twelve cell layers of thick-walled phellem, and only one layer of phelloderm. Grente and Berthelay-Sauret (37) described a "suberophellodermal generative layer" formed in bark of Castanea spp. in response to infection by a hypovirulent strain of E. parasitica. Cankers produced by this strain are quickly delimited, and advance of the mycelium is arrested.

Differences in wound periderm formation have also been related to the resistance or susceptibility of Pinus strobus L. to Cronartium ribicola F. (84). Trees which were resistant to fungal invasion had well developed wound periderm, while trees exhibiting only partial resistance had wound periderm which was incompletely developed. Trees classified as susceptible to the disease apparently formed no wound periderm after inoculation. Struckmeyer and Riker (84) also indicated that the phellogen of the wound periderm centrifugally produced alternate layers of phellem cells and lignified sclerenchyma cells, with each layer being several cells in thickness. Other anatomical aspects of the parasitism of phelloderm and other bark tissues of P. strobus by C. ribicola are provided by Hirt (39).

Marks and Minko (55) investigated wound periderm formation in Pinus radiata D. Don in response to infection



by Macrophoma pinea (Desm.) Petrak & Syd. Wound periderm was observed to develop within three to nine days after mechanical injury, and such wounds also became resistant to infection after the same period of time had elapsed. Evidence is provided which indicates that wound periderm develops more rapidly at temperatures of 25 C than it does at 15 C (55).

Wound periderm development in Populus tremuloides Michx. in response to infection by Macrophoma tumefaciens Shear is reported to occur rapidly enough to limit this pathogen to the outer bark region (48). Apparently, the fungus is able to penetrate through the wound periderm very slowly. When the phellogen is reached at last, a new wound periderm is generated from tissues deeper within the bark. Infection results in giving a rough-barked appearance to the tree (48). The response of bark tissues of P. balsamifera Muenchh. to infection by this pathogen has been detailed by Zalasky (99).

Hypocotyls were used to study the ontogeny of wound periderm formation in Fraxinus pennsylvanica (11). Periclinal cell divisions in the cortex appeared as soon as 60 hours after wounding. Normal and wound periderms were confluent within 30 days after injury. It was later observed that when a concentration of 100 ppm of indole-acetic-acid, naptalam, or 2,4-D was applied to injured hypocotyls, development of normal periderm was retarded (16). The application of up to 1000 ppm of these same growth

regulating substances did not retard wound periderm formation, indicating differences in physiological control between the two periderm types (16).

Grant and Spaulding (36) reported that several cork layers were produced in branches of Acer rubrum L. which had been previously inoculated with Nectria spp. All layers had been unsuccessful in arresting pathogen invasion with the possible exception of the last-formed layer (36). Further evidence is presented which indicates that bark injuries made in the fall are more conducive to Nectria canker development than injuries made during other seasons (4, 36). Zalasky (100) has indicated that Nectria galligena Bres. is able to penetrate mature periderm of young Populus tremuloides and Salix amygdaloides Anderss. trees. Most infections, however, were limited to the cortical region of the stem by a suberized wound periderm.

Investigations on the relationship of wound periderm formation to the development of beech bark disease of Fagus sylvatica in Europe have been recently provided (18, 19, 33). Braun (18) has given a detailed description of the general bark structure of F. sylvatica. Characteristic of mature bark is the presence of numerous, large sclerotized phloem rays. Feeding on bark parenchyma by the scale insect Cryptococcus fagisuga results in bark damage and stimulates a wound periderm to form (19). The presence of the sclerotic phloem rays, however, is reported to prevent the wound periderm from forming an uninterrupted

barrier to the subsequent invasion by Nectria coccinea (18). Bark is also subject to cracking along the interface of the phloem ray and the wound periderm (18).

During the last 15 years, a series of papers published by Mullick and coworkers has greatly expanded our understanding of the physiology of woody plant periderms in general (57, 58, 62), and of wound periderms in particular (60, 63, 64, 67). This work has been summarized in a detailed review (61).

Investigations of the wound periderms formed in the bark of Abies spp. as a result of feeding injury by the balsam woolly aphid, Adelges piceae Ratz., led Mullick to the following discovery. Some sequent periderms were found to be fundamentally identical to first periderms, while most sequent periderms were found to be fundamentally identical to periderms which arose in response to mechanical injuries, insect injuries, or pathogen invasion (59, 62, 63). In light of this discovery, the terms "first" and "sequent" periderms were no longer descriptive of the periderm types.

New terminology was proposed by Mullick and Jensen (63) whereby wound periderms and normally developed sequent periderms identical to them were termed necrophylactic periderms. First periderms and sequent periderms identical to them were termed exophylactic periderms. Exophylactic periderms could then be described as either first exophylactic or sequent exophylactic, depending on their time of origin (63).

The development of necrophylactic periderm was found to depend on the formation of a previously undescribed tissue designated as non-suberized impervious tissue (NIT) (60). The development of NIT was observed to occur in a zone which delimited sites of mechanical injury or pathogen invasion. Once this zone formed, healthy bark tissue was effectively separated from the invaded or dead bark tissues. A necrophylactic periderm was then able to differentiate from the living bark tissues abutting the NIT zone (60, 67).

The occurrence of necrophylactic periderms has been described for over 40 species representing 14 genera of conifers (47, 59), and for six genera of deciduous tree species (7, 82). Based on his studies, Mullick (61) was led to conclude that the development of a necrophylactic periderm in response to mechanical injury or pathogen invasion was a non-specific, autonomous defense reaction. Necrophylactic periderms which developed and successfully arrested pathogen ingress were suggested to be the result of a more basic resistance mechanism, and not the fundamental cause for the resistance (61).

## CHAPTER II

### ONTOGENY OF EXOPHYLACTIC PERIDERM AND GENERAL BARK STRUCTURE OF AMERICAN BEECH

#### Introduction

Studies of first periderms in woody plants have been made for a large number of taxa (83). Perhaps the most well-studied aspect of periderm development is the site of phellogen initiation (12, 22, 28). Mullick and Jensen (63) have recently shown that most sequent periderms are chemically and physically different than first periderms, and have proposed the term "exophylactic" to describe the first-formed periderms and sequent periderms identical to them. A few other reports have detailed the early developmental stages of periderm ontogeny (2, 14, 15), but documentation regarding site of phellogen initiation and subsequent periderm development of American beech (Fagus grandifolia) is apparently lacking.

Several reviews of general bark structure of woody plants have been presented (8, 31, 83). Anatomical aspects of bark of European beech (Fagus sylvatica) have been detailed by Whitmore (97) and Braun (18). Although statements appear in the literature (18, 32) indicating that the bark anatomy of European beech is very similar to that of American beech, no documentation has been found which deals specifically with the anatomical aspects of the bark

of American beech.

The objectives of the present study were to (i) determine the site of phellogen initiation, (ii) describe the first year ontogeny of the first exophylactic periderm in current year shoots of American beech growing under natural conditions, and (iii) describe the general appearance and structure of mature beech bark.

#### Materials and Methods

Five current year shoots were obtained at each collection period from each of two mature (20 and 64 cm diameter) American beech trees growing in a natural woodland at the Kingman Research Farm in Madbury, New Hampshire. Shoots were collected at weekly intervals from mid-April through June, biweekly intervals from July through September, and monthly intervals from October through December, 1980.

Several sections 1 to 2 mm in length were cut from the midsection of each shoot, fixed in Karnovsky's fixative, dehydrated through a gradual ethanol series ending with 100% propylene oxide, infiltrated with and embedded in Spurr's epoxy resin (50). Transverse and longitudinal sections 3 to 5  $\mu$ m in thickness were cut from the embedded tissues using an ultramicrotome equipped with a glass knife. Sections were mounted on standard glass microscope slides and stained with either Toluidine Blue O, Sudan Black B, IKI, or left unstained (65). Tissue sections were then examined microscopically.

Mature bark of American beech growing in natural forest

stands was examined at the University of New Hampshire Kingman Research Farm in Madbury, New Hampshire, the Bartlett Experimental Forest near Bartlett, New Hampshire, and at the Hubbard Brook Experimental Forest near West Thorton, New Hampshire. The latter two areas are administered by the USDA Forest Service. Numerous bark samples were cut from trees at all three research locations during the period from July, 1979 to March, 1982. Microscopic examinations of mature beech bark were made by sectioning 10 to 20  $\mu$ m thick sections using a commercial cryostat set at a cutting temperature of approximately -25 C. Sections were either stained with Toluidine Blue O or left unstained, and mounted in glycerol on standard glass microscope slides. Other bark samples were cut into pieces 1 X 2 mm, embedded in Spurr's epoxy resin, and prepared as described for current year shoots.

### Results

#### Phellogen Initiation and Exophylactic Periderm Development.

Time of budbreak between individual American beech was found to occur over a period of approximately ten days. The approximate time of budbreak for American beech trees growing at the Kingman Research Farm occurred from April 28 until May 6 during 1980. Trees of which current year shoots had already elongated up to 6 cm were often found within a few meters of trees with buds just beginning to expand. Phellogen initiation and periderm development began very soon after shoots had started to elongate. The time of

occurrence of developmental events for the sampled shoots, however, never varied between trees over more than one collection period.

Initiation of the phellogen occurred in the sub-epidermal layer of the cortical cells as early as May 5, within one week after budbreak (Figs. 1, 2). At first, the phellogen was not continuous around the shoot, but became so within a week of initiation. The second cell division of the phellogen had occurred by the second week of shoot growth (Fig. 3). Anticlinal as well as periclinal divisions were also evident at this time, and throughout the remainder of the year. The daughter cell of the first division centripetal to the stem axis remained meristematic, while the other developed into a phellem cell. Subsequent development of the periderm occurred in this centripetal fashion until mid-June. Collapse of the epidermal cells occurred in late May and early June (Fig. 4).

The phellem which had developed by early July was five to six cell layers in thickness (Fig. 5), and few if any additional cell layers were added during the remainder of the year (Figs. 6-12). Phellem cells became suberized very soon after being formed (Figs. 13, 14). The walls of the phellem cells were approximately 1.2  $\mu\text{m}$  thick at maturity. Tangential interior and exterior and radial walls appeared uniform in thickness. Phellem cell shape appeared to change slightly during aging. Newly formed phellem cells were approximately 5 X 6  $\mu\text{m}$  in cross section. Older phellem cells



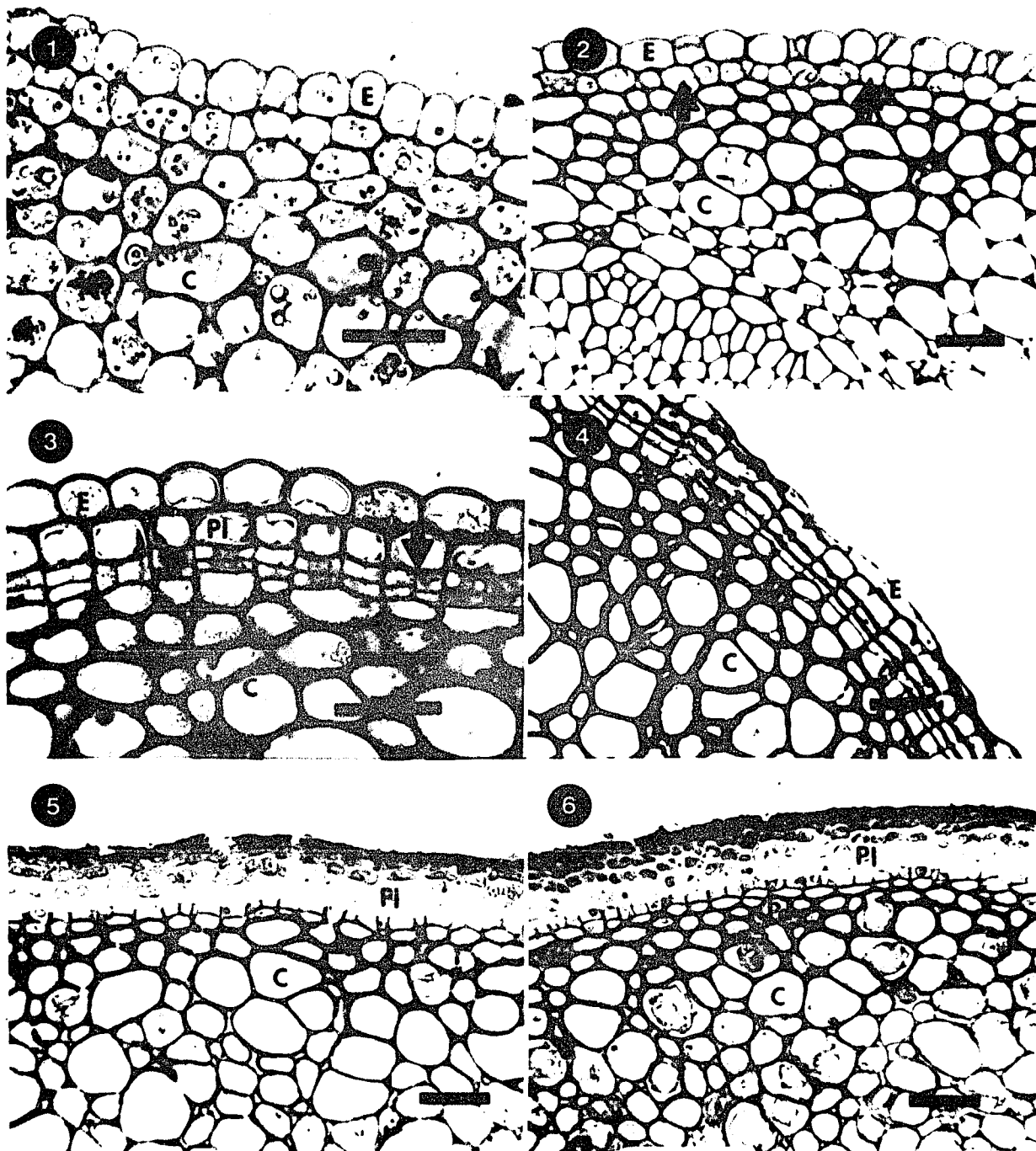
Figures 1 - 6. Exophylactic periderm development in American beech May 1 to July 28, 1980.  
Fig. 1. Undifferentiated cortex and epidermis, early May.  
Fig. 2. Phellogen initiation in subepidermal cortical cell layer, May 5 (arrows). Phellogen is not yet continuous around the circumference of the stem. Fig. 3. Periderm development as of May 13. Anticlinal (arrow) as well as periclinal divisions have occurred. Fig. 4. Third centripetal division of the phellogen, June 4. Collapse of the epidermal cells is now apparent. Fig. 5. Periderm development as of July 14. Five cell layers of phellem are evident. Fig. 6. Periderm development as of July 28. Five to six cell layers of phellem and one discontinuous cell layer of phelloderm are evident.

Scale bars in Figures 1-4 = 22  $\mu\text{m}$ .

Scale bars in Figures 5 and 6 = 50  $\mu\text{m}$ .

All Figures depict transverse sections.

For all Figures: C = cortex, E = epidermis, Pd = phelloderm, Pl = phellem.



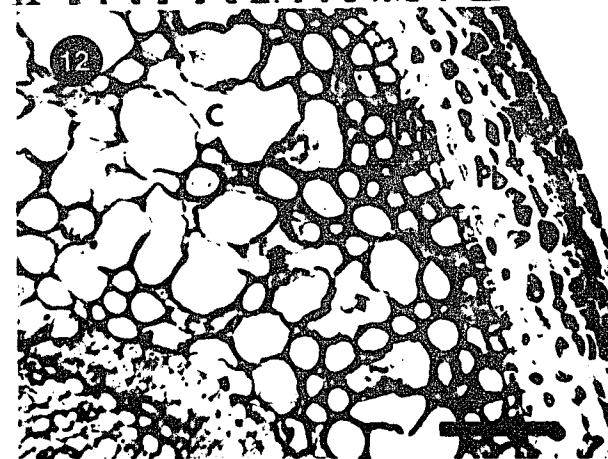
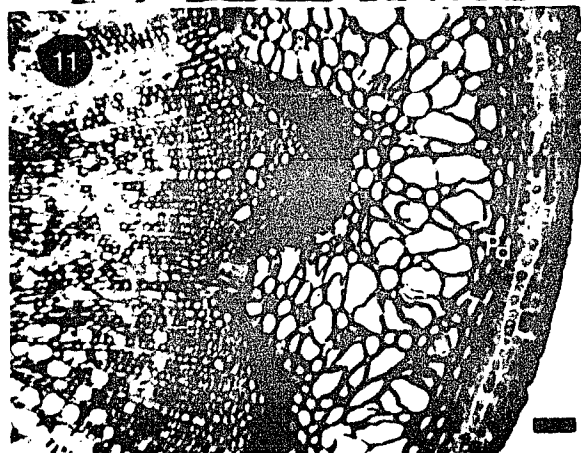
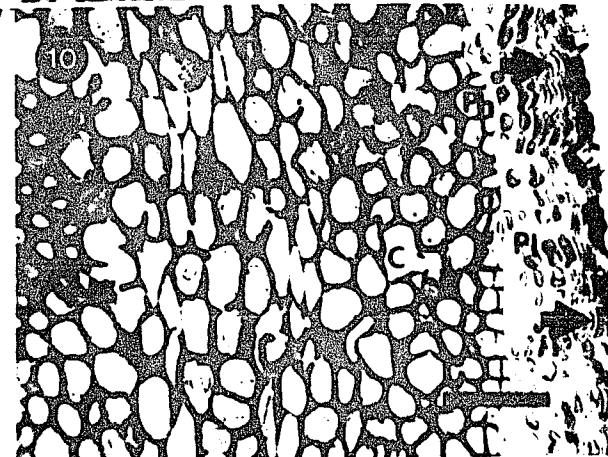
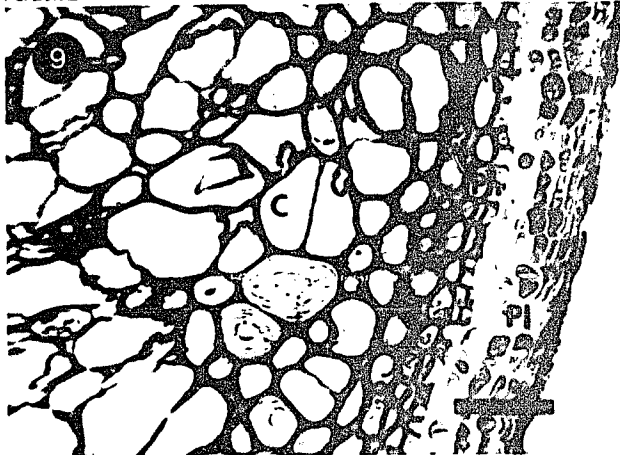
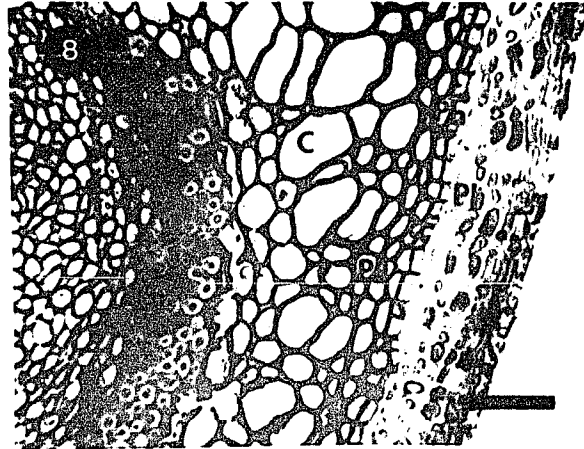
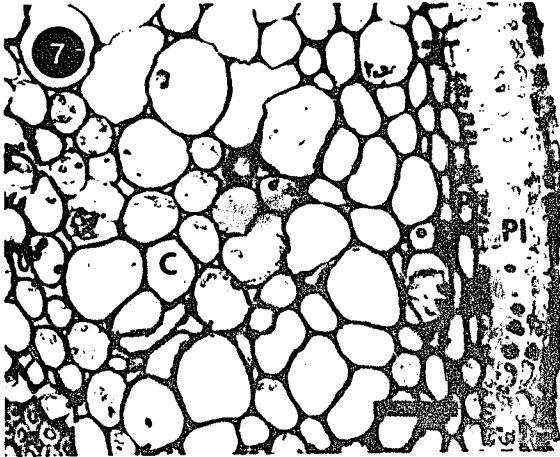
Figures 7 - 12. Exophylactic periderm development in American beech August 11 to December 29, 1980.

Fig. 7. Periderm development as of August 11. Fig 8. Periderm development as of September 8. Note the well developed phelloderm one to two cell layers in thickness. Fig. 9. Periderm development as of September 22. Fig. 10. Periderm development as of October 20. Note tangential stretching of outer phellem cell layers (arrows). Fig. 11. Periderm development as of November 20. A well defined phelloderm is evident. Fig. 12. Periderm development as of December 29. Note that the number of phellem cell layers (five to six) is the same as that for tissues collected in July (Fig. 5).

Scale bar in each Figure = 50  $\mu$ m.

All Figures depict transverse sections.

For all Figures: C = cortex, Pd = phelloderm, Ph = phellogen, Pl = phellem.



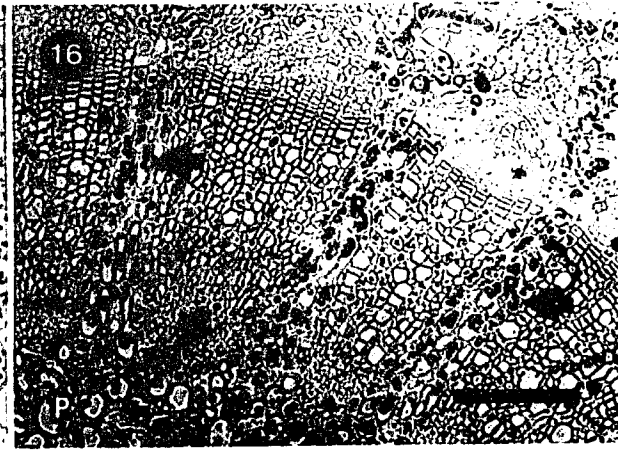
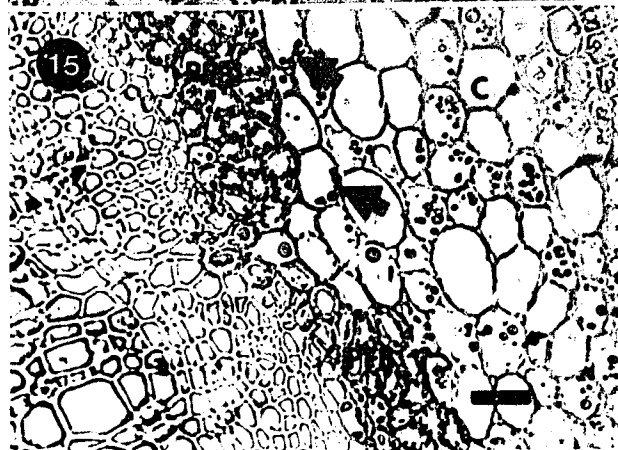
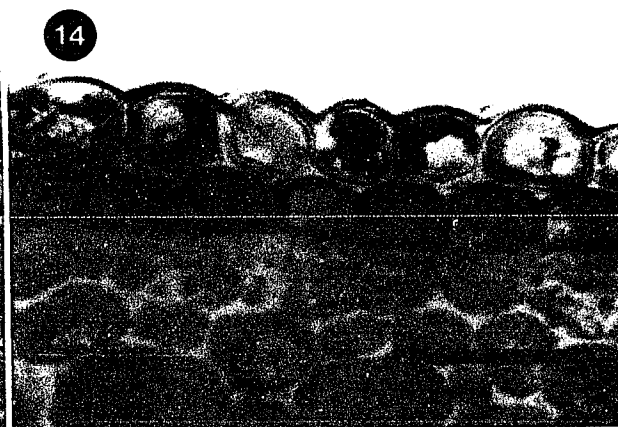
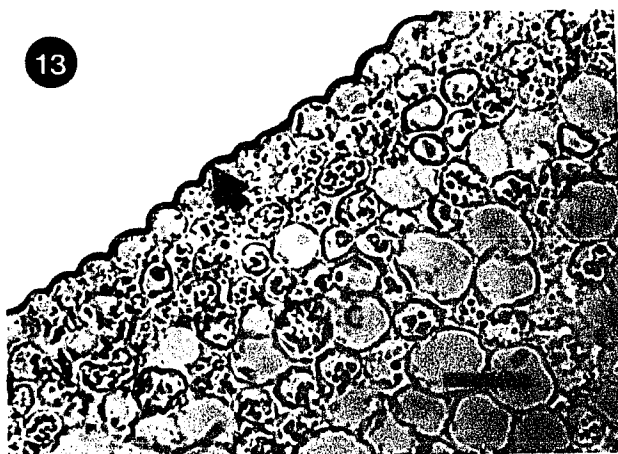
Figures 13 - 16. Suberin and starch in current year shoots of American beech. Fig. 13. Cuticle (arrow) darkly stained with Sudan Black B, indicating presence of suberin, from tissue collected May 5, 1980. Fig. 14. Rapid suberization of newly developed phellem cells is indicated by darkly stained cells. Tissue collected May 13, 1980. Fig. 15. A few starch grains (arrows) are evident in this tissue collected in early May and stained with IKI. Starch appeared only in a few cortical cells which bordered on phloem fiber bundles. Fig. 16. By November 20, starch accumulation in cells of rays and pith (arrows) of current year shoots was evident. Starch grains were never observed in periderm tissues, regardless of the time tissue was collected.

Scale bars in Figures 13 to 15 = 28  $\mu\text{m}$ .

Scale bar in Figure 16 = 75  $\mu\text{m}$ .

All Figures depict transverse sections.

For all Figures: C = cortex, P = pith, PFB = phloem fiber bundle, Ph = phellogen, Pl = phellem, R = ray.



became tangentially stretched, with cell dimensions approximating 3 X 8  $\mu$ m.

Phelloderm development was difficult to study because of the relative similarity of cortical parenchyma cells to phelloderm cells. The primary characteristic which allowed distinction between phelloderm and cortical parenchyma cells was cell size. Phelloderm cells were, in general, smaller than cortical parenchyma cells, but were not arranged in a continuous or uniform layer, as were those of the phellem. A continuous layer of phelloderm cells had apparently developed by late July (Fig. 6). A distinct phelloderm was observed in shoots collected in November, seven months after budbreak (Fig. 11). The phelloderm of individual shoots at this time varied in thickness from one to two cell layers.

No starch grains were observed in the phelloderm of tissue sections cut from shoots collected in July. A few starch grains were present in cortical parenchyma adjacent to phloem fibers (Fig. 15). Numbers of starch grains gradually increased through the year until fall when ray, pith, and some cortical parenchyma cells appeared packed with the (Fig. 16). Phelloderm cells, however, remained free of any starch.

#### General Appearance and Structure of Mature Bark.

Observation of numerous American beech revealed the presence of three distinct bark patterns. Generally, the bark of beech appears smooth and tight

relative to that of other northern hardwood tree species. Lenticels are numerous and quite conspicuous (Figs. 17, 18). Although variations in bark appearance have been observed (Figs. 19-22), the first exophylactic periderm remains in a superficial position throughout the life of the tree in most cases (Fig. 23). This periderm consists of a phellem 20 to 35 cell layers in thickness, a single phellogen layer, and one to two cell layers of phelloderm (Fig. 24).

The bark is characterized by the presence of sclerified phloem rays which vary in size from 50 to 100  $\mu\text{m}$  in width (radially) and from 0.75 to 3 mm or more in length (axially). These sclerified phloem rays appear to project into the xylem (Fig. 25). Numerous, smaller, nonsclerified phloem rays are also present, and appear in cross section as an undulating or zig-zag pattern. Also present throughout the bark are large groups of sclerenchyma cells.

Total bark thickness is strongly correlated with tree diameter (Fig. 29). Bark thickness of the largest trees measured in this study ranged from 4.6 to 6.3 mm. Periderm thickness, however, is not strongly correlated with either tree diameter or bark thickness (Figs. 30, 31). Once the periderm has reached approximately 0.2 mm in thickness, a stabilization becomes apparent.

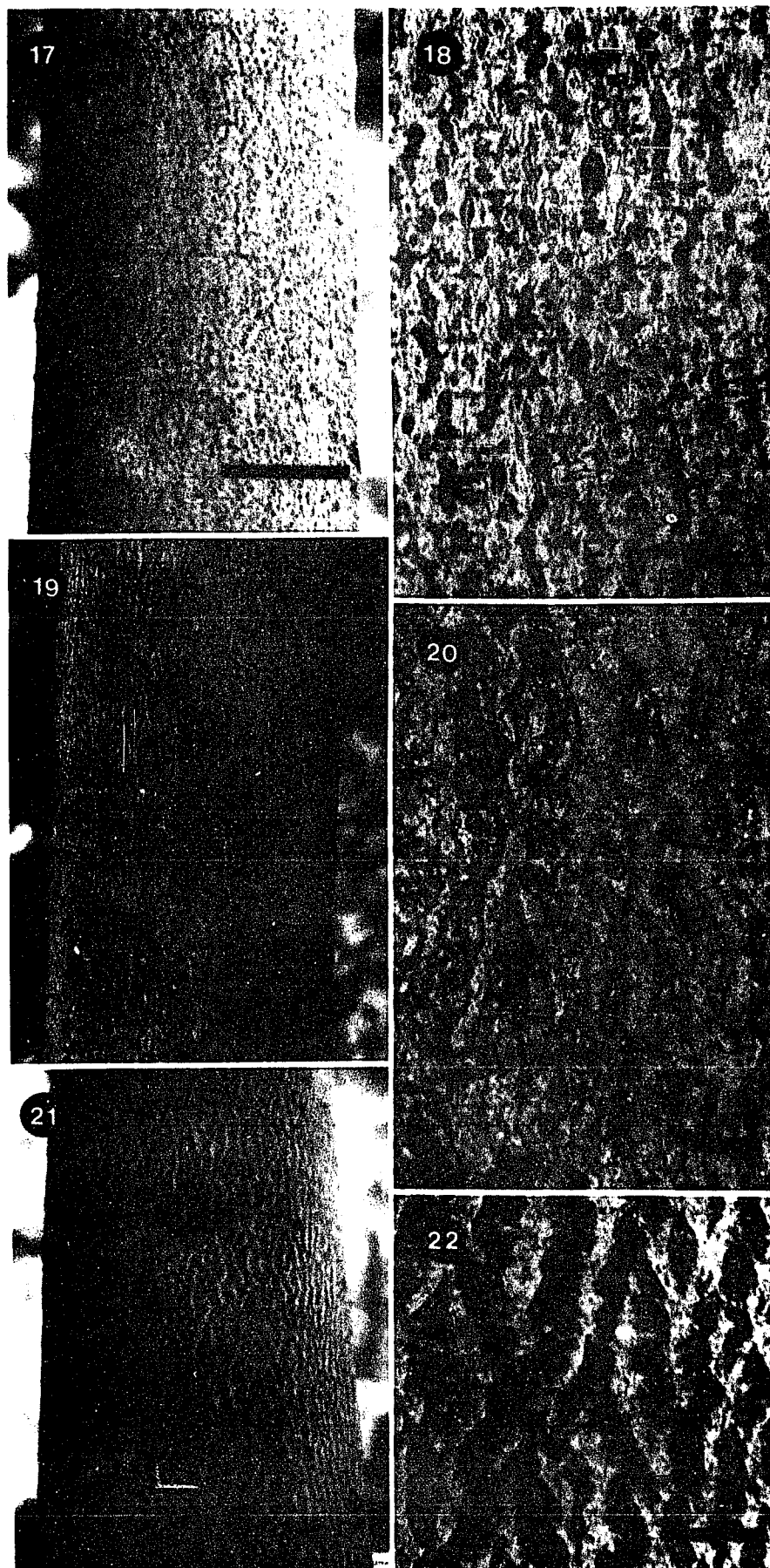
Two variations of the normal bark patterns were observed in the course of this study (Figs. 19-22). The first variation was exhibited by several saplings and pole-sized trees at the Kingman Research Farm and at the Hubbard Brook



Figures 17 - 22. Bark types of American beech.  
Figs. 17 and 18. Typical smooth bark appearance of American beech. Figs. 19 and 20. "Curly" bark pattern found on several pole-sized trees. Exfoliation of the phellem resulted in shallow fissures. Figs. 21 and 22. Deeply fissured bark pattern found on a single tree. This bark pattern was not confined to a small region of the stem, but was uniform around the stem circumference to a height of at least 6 m.

Scale bars in Figures 17, 19, and 21 = 7 cm.

Scale bars in Figures 18, 20, and 22 = 5 mm.



Figures 23 - 28. Periderm development in relation to bark types of American beech. Fig. 23. General appearance of typical smooth bark. Thin superficial periderm (small arrows) and demarcation between "expansion" tissue and "hard bast" (large arrow) is shown. Fig. 24. Appearance of the first exophylactic periderm of bark sample shown in Fig. 23. Fig. 25. Appearance of sclerified phloem ray in relation to the vascular cambium. Fig. 26. Appearance of exophylactic periderm from trees with curly bark pattern. Note extensive development of phelloderm, and exfoliation of phellem (arrow). Fig. 27. Appearance of periderms from the tree with deeply furrowed bark pattern. Up to three distinct periderms were present, resulting in the development of a true rhytidome (large arrows). Note also the sclerified phloem rays (small arrows). Fig. 28. Section of bark shown in Fig. 27. Approximately 35 cell layers of phellem were present in each periderm layer of the rhytidome. No more than two cell layers of phelloderm (arrows) were observed in any single periderm.

Scale bars in Figures 23 and 27 = 5 mm.

Scale bar in Figure 24 = 150  $\mu$ m.

Scale bars in Figures 25, 26, and 28 = 200  $\mu$ m.

All Figures depict transverse sections.

For all Figures: Pd = phelloderm, Pl = phellem,

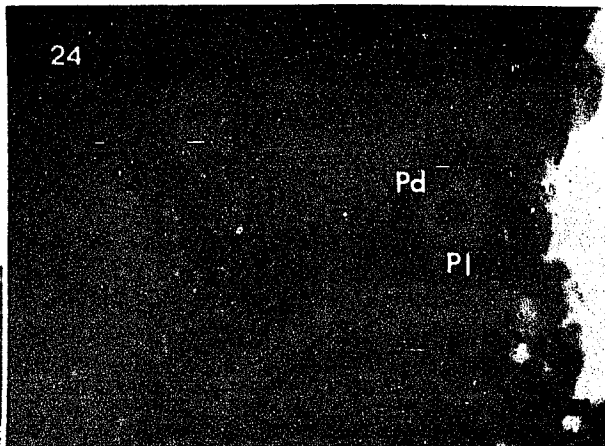
S = sclerenchyma, SPR = sclerified phloem ray,

VC = vascular cambium, X = xylem.

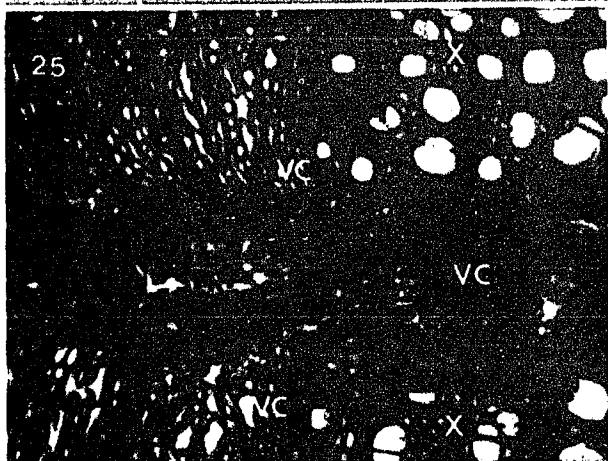
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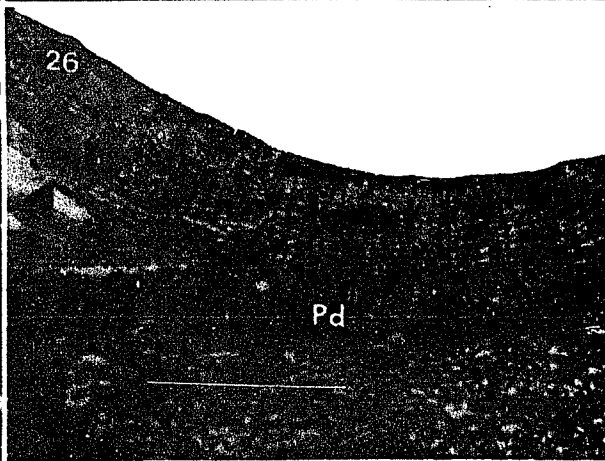
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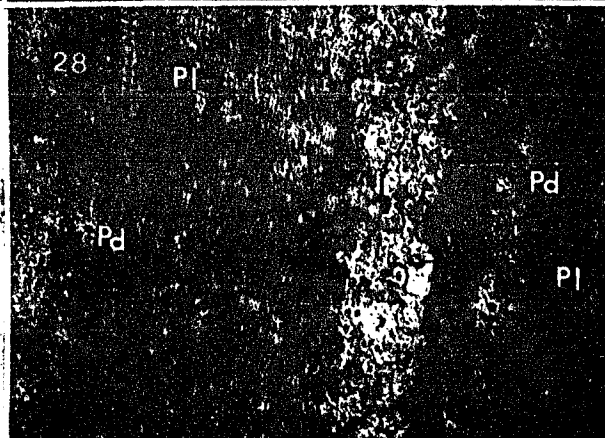


Figure 29. Relationship between tree diameter and bark thickness of American beech. Regression line has an  $r^2$  value of .92.

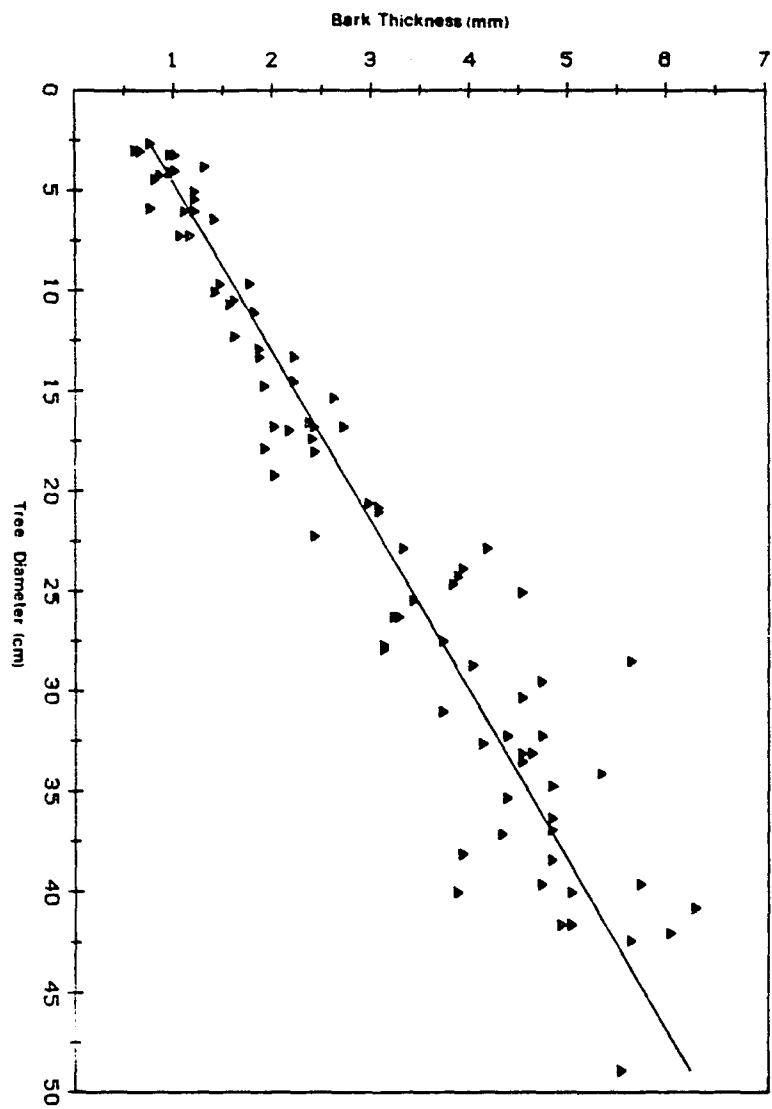
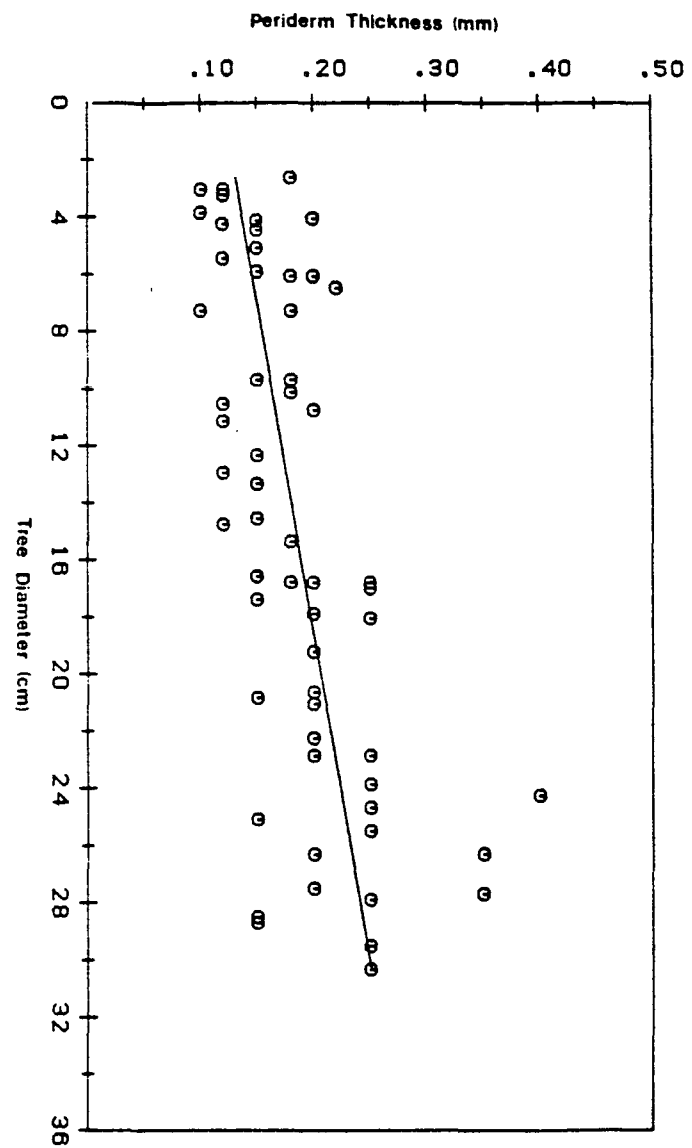
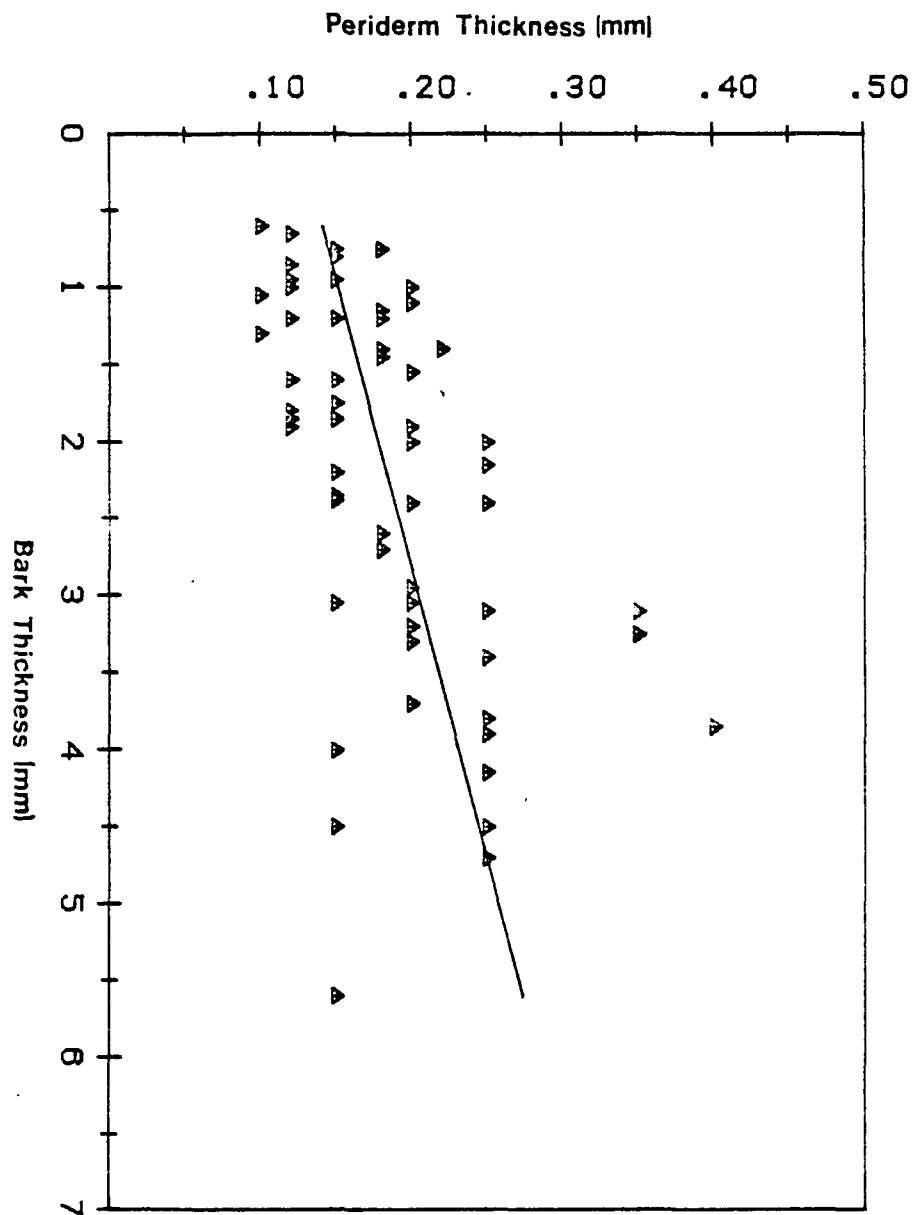


Figure 30. Relationship between tree diameter and periderm thickness of American beech. Regression line has an  $r^2$  value of .42.

Figure 31. Relationship between bark thickness and periderm thickness of American beech. Regression line has an  $r^2$  value of .39.





Experimental Forest. The second was exhibited by an individual tree at the Kingman Research Farm.

The first variation was characterized by exfoliation of bark tissues in long, shallow, curled ridges, ultimately resulting in a "curly" bark appearance (Fig. 20). Bark samples taken from these trees and sectioned for microscopic examination revealed that only phellem tissue was exfoliating (Fig. 26). The curls of bark which were separating from the tree bole were 15 to 20 phellem cell layers in thickness. This accounted for approximately one half of the total phellem thickness. The phellogen consisted of a single cell layer. The phelloderm, however, appeared to be more developed in curly-barked trees than in trees exhibiting the more common, smooth-barked appearance. Phelloderm in curly-barked trees was four to five cell layers or more in thickness (Fig. 26).

Another variation in bark appearance was characterized by deep, regular furrows and ridges (Fig. 22). This bark resembled that of other hardwoods such as species of Acer and Quercus, which normally develop sequent periderms. The individual exhibiting this bark pattern was growing on a slight incline, the bole being slightly "J" shaped near the ground. The bark pattern, however, was consistent around the circumference of the bole from approximately 20 cm above the ground to well above 3 m (Fig. 21). The bark from the ground to 20 cm was deeply fissured on the uphill side of the bole, and less so on the downhill side.

Bark samples obtained from several locations on this tree and sectioned for microscopic examination revealed the presence of up to three distinct periderms. This tree had developed a true rhytidome (Fig. 27). Phellem layers of these periderms numbered from 25 to 38. The phelloderm consisted of two cell layers generally, but was sometimes absent (Fig. 28).

### Discussion

Budbreak in American beech was found to vary between individuals over a period of approximately ten days at the Kingman Research Farm site. This variability is apparently due in large part to differences in genotype. This is supported by the fact that trees of similar age and size growing in close proximity to one another had distinctly different budbreak dates.

The phellogen is initiated in the subepidermal layer of cortical parenchyma. This site of initiation is the same as that reported for European beech by Duliott (28). Phellem development is centripetal in nature for at least the first four or five divisions of the phellogen. This is to say that the daughter cell resulting from the division of a phellogen cell which remains meristematic is the daughter cell closest to the stem axis. Phelloderm first appears as individual cells, and not as a continuous cell layer until most or all of the phellem cell layers are formed for that first growing season.

Periderm development in current year shoots, therefore,

appears to occur in two overlapping phases: a phellem development phase followed by a phelloderm development phase. The phelloderm development phase is not completed until the phellem development phase is completed, or nearly so. Whether these two growth phases continue as distinct processes in the periderm of older stems is unknown.

Generally, phellogen activity is relatively slow compared with activity of the vascular cambium (51). Waisel et al. (95) reported that the vascular cambium of Robinia pseudoacacia may develop five times the number of xylem derivatives in a single month as the number of derivatives which the phellogen produces in a year. The ratio of vascular cambium derivatives to phellogen derivatives in American beech was found to be somewhat lower. Twelve to fourteen xylem cell layers were produced, on average, to the six phellem and two phelloderm cell layers produced by the phellogen by the end of the first growing season.

One of the most striking aspects of periderm development in American beech is the rate at which the process occurs. Periderm development starts within a few days after initial shoot elongation begins, and phellem development is essentially completed by mid-June. The completion of this process so early in the growing season also coincides with time of budset (6). Bicknell (6) suggests that rapid and early development of American beech in terms of budbreak, shoot elongation, and leaf expansion provides a competitive advantage to trees growing in the

forest understory, and may contribute to their ability to survive as a shade-tolerant species.

The general structure of mature bark of American beech appears to be similar to that reported for European beech (18). Braun (18) has distinguished four principal regions in European beech bark: the soft bast, the hard bast, the primary bark, and the surface periderm. The primary bark region coincides with the expansion tissue described by Whitmore (97). All four regions may be observed in American beech bark as well. Small parenchymatous phloem rays and larger, totally sclerified phloem rays characteristic of European beech bark are also present in American beech bark.

Bark thickness in American beech is shown to be highly correlated with stem diameter. The thickness of the periderm, however, remains nearly constant regardless of stem diameter. This may be due, in part, to the fact that in order to remain in a superficial position, a great deal of energy of the phellogen is utilized during anticlinal divisions. The number of periclinal divisions a phellogen cell undergoes may be limited by the number of anticlinal divisions it must undergo.

Although no reports have been found which describe the curly bark pattern observed in this study, the deeply fissured bark pattern has been observed elsewhere (69). However, an anatomical explanation of this bark pattern was not provided by the report. Braun (18) has also indicated that very large, old European beech sometimes develop a thin

rhytidome on the lower stem. He indicated that the inner periderm can form because the bark tissue from which it is differentiated lies beyond the reach of the large, sclerified phloem rays. Both the earlier report (69) and the present investigation of rhytidome development in American beech indicate that this bark pattern is not confined to the lower stem region or to very old individuals. The degree to which this characteristic is under genetic control is unknown.

### CHAPTER III

#### NECROPHYLACTIC PERIDERM DEVELOPMENT IN CURRENT YEAR SHOOTS OF AMERICAN BEECH

##### Introduction

Few studies of the developmental stages of necrophylactic, or wound periderm formation in woody plant species have been reported. However, based on what is known, it appears that certain biological and environmental factors can greatly influence this developmental process. A greater understanding of the factors which influence host response to mechanical damage or pathogen invasion is desirable, since it may lead to the evolution of new or more effective control techniques.

The wounding of hypocotyls of Fraxinus pennsylvanica which had not yet developed a normal first periderm was reported to result in the initiation of a wound periderm within 60 hours (11). Borger and Kozlowski (11) also reported that development of wound periderm stimulated formation of the first periderm in these hypocotyls. Marks and Minko (55) have indicated that temperature influences rate of development of wound periderms in Pinus radiata infected by Macrophoma pinea. Necrophylactic periderm development in young stems of Populus maximowiczii X trichocarpa has been recently reported (7). Necrophylactic periderm developed in the cortex around wounds which were

either inoculated with Cytospora chrysosperma Pers. ex Fr. or left uninoculated.

Mullick and Jensen (64) have provided evidence indicating that rates of necrophylactic periderm development in mature bark of several conifer species is largely dependent on season of wounding. They reported that the time interval between wounding and formation of a non-suberized impervious tissue, the development of which is prerequisite to necrophylactic periderm formation (60), was shortest (14 days) when wounds were inflicted in late spring, gradually increased through late fall (35 days), and was longest in winter (over 70 days).

The primary objective of the present study was to describe and compare the rate of necrophylactic periderm development in current year shoots of American beech (Fagus grandifolia) after wounding in spring with rate of development after wounding in fall. A second objective was to determine the effect of inoculating wounds with Nectria coccinea var. faginata on rates of necrophylactic periderm formation in spring and fall.

#### Materials and Methods

##### Fieldwork.

Ten American beech trees, each approximately 2 cm in diameter at ground level, were selected at the Kingman Research Farm in Madbury, New Hampshire. Current year shoots on five of the trees were used for the spring experiment, and the remaining five for the fall experiment.

Forty current year shoots were randomly selected on each of five trees on May 28, 1980. A single treatment was applied to each of ten shoots on each tree. Shoots were either wounded, wounded and inoculated with Nectria coccinea var. faginata, left unwounded but inoculated, or left unwounded and uninoculated.

Wounds were inflicted by lightly making a single scratch through the shoot epidermis from the most recent budscale scar to the shoot apex with a sterilized dissecting needle. Inoculum consisted of a spore suspension of  $2.4 \times 10^5$  microconidia of N. coccinea var. faginata per ml of sterile distilled water. The suspension was made with spores from a three week old malt agar culture of the fungus (88). The isolate used was a single-ascospore isolate obtained from a natural bark canker at the Hubbard Brook Experimental Forest near West Thorton, New Hampshire.

One shoot representing each treatment from each of the five trees was collected at each sampling date. Sampling dates included one immediately following wounding on 28 May, 1980 and the others 1, 2, 3, 4, 6, and 8 weeks thereafter.

This same experimental design was applied to five additional trees for the fall experiment. Shoots were wounded, wounded and inoculated, inoculated, or left as controls on 25 September, 1980. One shoot representing each treatment from each of the five trees was collected at each sampling date. Sampling dates again included one immediately following wounding, and the others 1, 2, 3, 4,



6, and 8 weeks thereafter.

### Microtechnique.

Sections 1 to 2 mm long were cut from shoots and immediately placed in Karnovsky's fixative (50), dehydrated through a gradual ethanol series ending with 100% propylene oxide, infiltrated and embedded in Spurr's epoxy resin (50). Tissue sections were then cut 2 to 4  $\mu$ m in thickness using an ultramicrotome equipped with a glass knife. Sections were stained in either Toluidine Blue O, Sudan Black B, IKI, or left unstained. Unstained material was examined with fluorescence microscopy using an American Optical Fluorolume fluorescence illuminator fitted with OG1, EK2A, and GG9 barrier filters and Corning No. 5840, 5113, and Schott BG-12 exciter filters.

### Results

#### Necrophylactic Periderm Development in Shoots Injured in Spring.

The injuries inflicted on shoots in spring varied somewhat in depth between shoots, but all except a few resulted in direct damage only to the epidermis and cortex. Occasionally the phloem fiber bundles were disrupted. Developmental events in the formation of a necrophylactic periderm appeared quite consistent between shoots collected after the same time intervals.

One week after wounding, a distinct necrotic region was apparent at the wound interface. This region was characterized by three to four layers of collapsed cortical

cells (Fig. 32). Well behind the necrotic region, in some cases up to ten cortical cell layers away, divisions in the cortical parenchyma were observed (Fig. 32). These divisions are interpreted as the initiation of the necrophylactic periderm. Divisions occurred in a plane parallel to the wound surface. The newly initiated phellogen of the necrophylactic periderm was not continuous around the wound zone at this time, but became so within two weeks after the initial wounding. Suberization of the daughter cells centrifugal to the stem axis had not yet occurred, as evidenced by lack of staining with Sudan Black B.

The appearance of wounded shoot sections was similar to sections from shoots which had been wounded and inoculated. Germinated microconidia were observed on the wound surface, and mycelium was present in the cortical cells of the first one or two layers of cells behind the necrotic zone (Fig. 33). This was the greatest extent to which the fungus was observed to have advanced throughout the remainder of the experiment.

Two weeks after wounding, the necrophylactic periderm appeared as a continuous layer behind the wound, and had become confluent with the exophylactic periderm. Two to ten derivatives of the phellogen of the necrophylactic periderm had developed, depending on cell position with respect to the wound surface (Fig. 34). Suberization of one or two cell layers was apparent when sections were stained with Sudan Black B or observed with fluorescent microscopy.

Figures 32 - 37. Necrophylactic periderm development in current year shoots of American beech wounded in the spring. Fig. 32. Seven days after wounding, initiation of a necrophylactic periderm is evident in cortical cells surrounding the necrotic zone (arrows). Fig. 33. Hyphae are observed invading cortical cells bordering the necrotic zone seven days after wounding and inoculation with Nectria coccinea var. faginata. Fig. 34. Two weeks after wounding, a continuous and well developed necrophylactic periderm has become confluent with the first exophylactic periderm (arrows). Fig. 35. Three weeks after injury, collapse of all cells centrifugal to the necrophylactic periderm has occurred. Fig. 36. Four weeks after wounding, the necrophylactic periderm appears and functions as the first exophylactic periderm. Fig. 37. Subsequent development of the necrophylactic periderm six weeks after the shoots had been wounded.

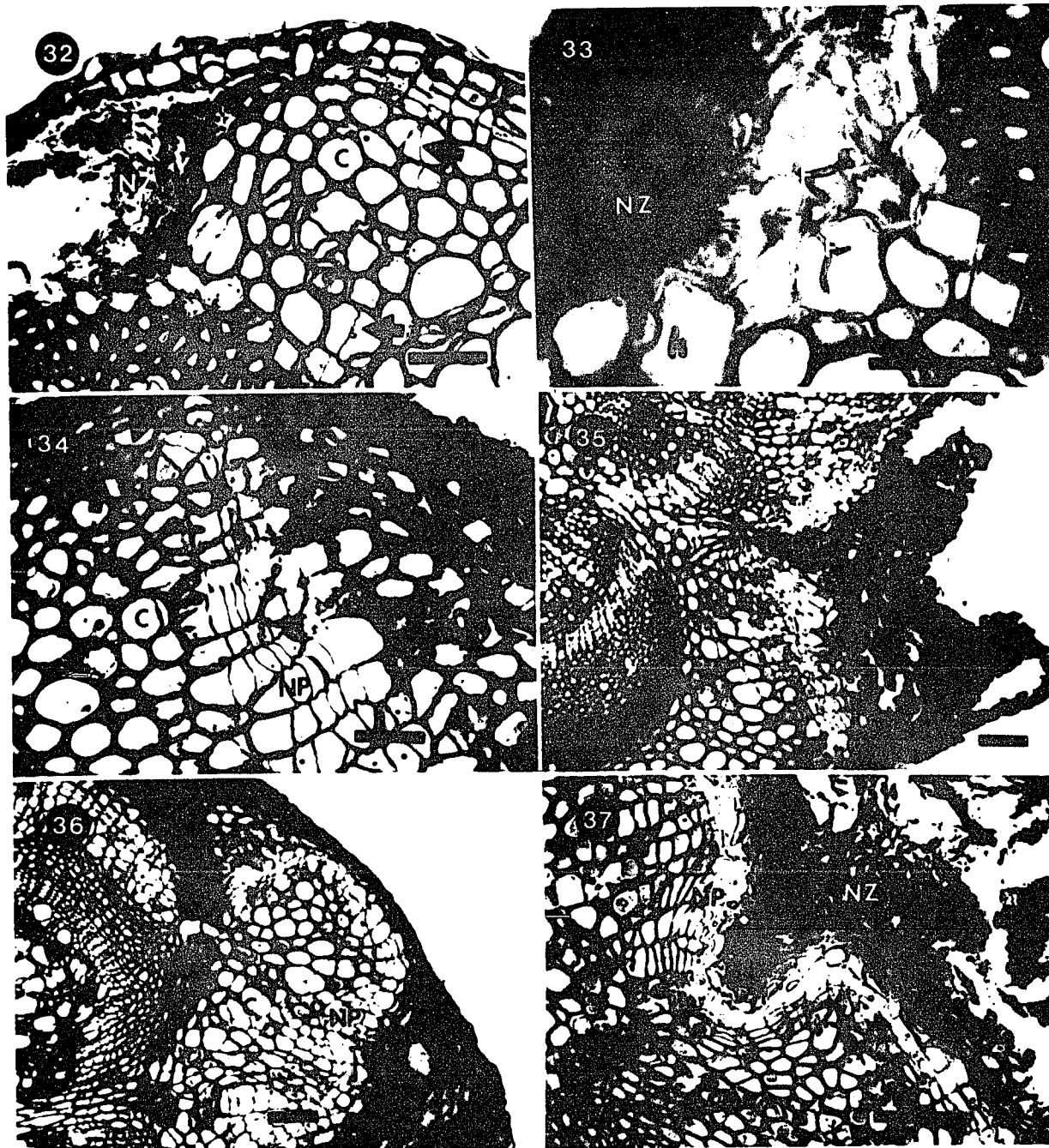
Scale bars in Figures 32 to 34 = 35  $\mu$ m.

Scale bars in Figures 35 to 37 = 70  $\mu$ m.

All Figures depict transverse sections.

For all Figures: C = cortex, F = fungus hypha,

NP = necrophylactic periderm, NZ = necrotic zone.



Necrophylactic periderm development continued in a manner similar to that described above until, three weeks after injury, a well-formed necrophylactic periderm with several cell layers of phellem was evident (Fig. 35). At this time, all cortical tissues outside the necrophylactic periderm had begun to collapse, or had already collapsed regardless of whether the wounds had been inoculated, or the cells had been damaged directly. Presumably, this was the result of the isolation of those cells external to the necrophylactic periderm from the living plant tissues. An examination of tissues collected four or six weeks after wounding revealed that the necrophylactic periderm appeared to function and develop in a manner similar to the first exophylactic periderm (Figs. 36, 37).

Shoots which were left uninoculated and unwounded did not appear different from those inoculated and unwounded with respect to exophylactic periderm development. The inoculated unwounded shoots did not become infected. There was no evidence that the fungus was able to penetrate the young exophylactic periderm in any of the sections examined.

Necrophylactic Periderm Development in Shoots Injured in the Fall.

Injuries inflicted on current year shoots in the fall appeared to be slightly more severe than those inflicted in the spring. In most instances, the phloem fiber bundles had been disrupted to some extent. The variation in wound severity between shoots was not considered significant.

Initiation of the necrophylactic periderm was not observed in sections of shoots collected one week after injury (Fig. 38), but was found in those collected after two weeks (Fig. 39). Not only were the cortical cells responding to the injury by dividing, but cells of the phloem beneath the phloem fiber bundles responded as well (Fig. 39). Hypertrophy of the dividing cells, but not of other cortical or phloem cells, was evident (Figs. 40, 41).

Two or three derivatives of the initiated but incomplete necrophylactic periderm were evident two weeks after injury. An examination of the injured shoots later in the season, at four, six, and eight weeks after injury, revealed that few if any additional cell divisions of the necrophylactic periderm had occurred (Figs. 42, 43). Also evident was the nonuniform alignment of the dividing cells, the divisions occurring in an apparently random fashion with respect to the plane of the wound surface (Fig. 41). Often the dividing cells themselves appeared not to be in a distinct zonal arrangement (Fig. 41). Staining sections with Sudan Black B indicated that suberization of the newly formed cells had not occurred, even as late as eight weeks after wounding. Again, as with the experiment conducted in the spring, no infections of wounded or unwounded tissues resulted in canker development.

#### Discussion

Current year shoots of American beech wounded in spring were able to initiate a necrophylactic periderm at least one

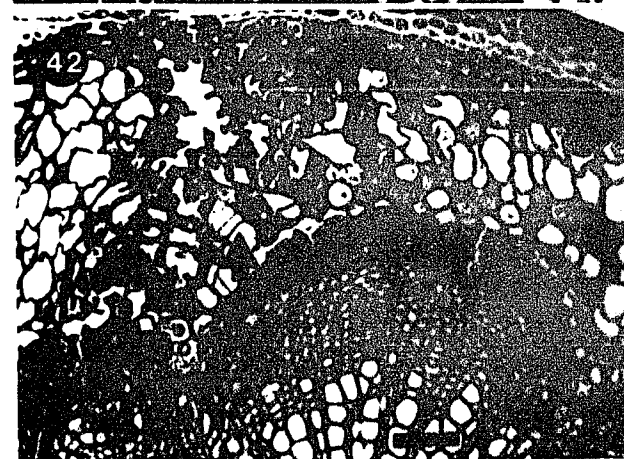
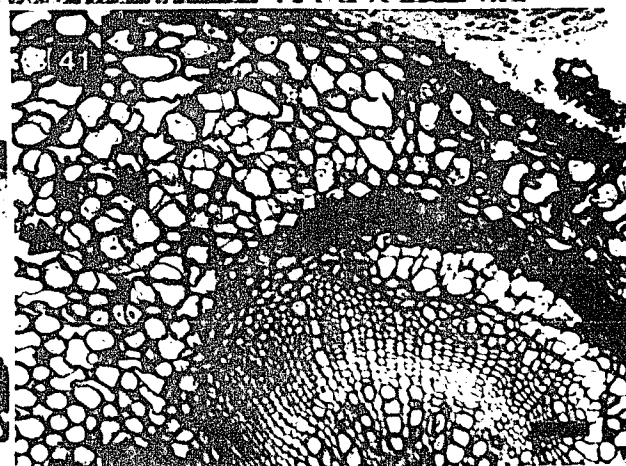
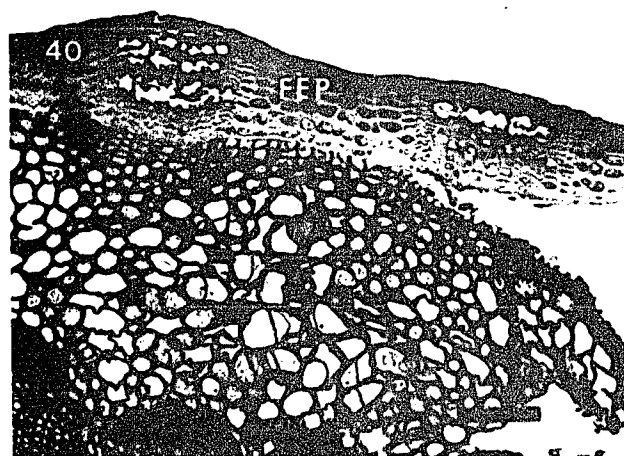
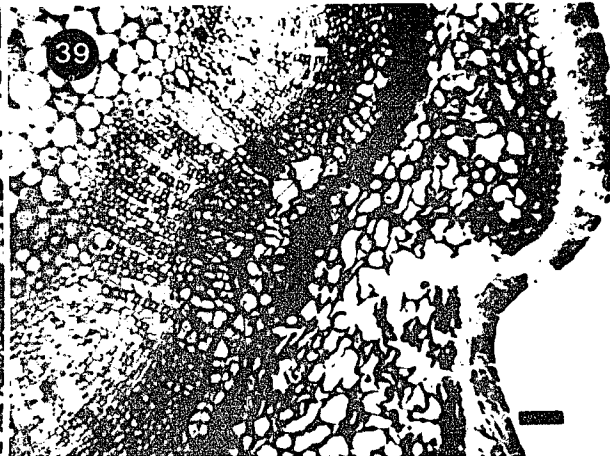
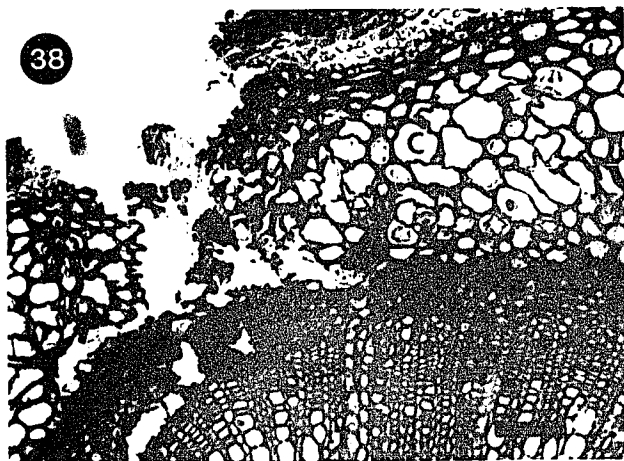
Figures 38 - 43. Necrophylactic periderm development in current year shoots of American beech wounded in the fall. Fig. 38. One week after wounding, no evidence of initiation of the necrophylactic periderm is apparent in cortical cells. Fig. 39. Two weeks after wounding, initiation of the necrophylactic periderm is evident in phloem cells (arrow). Note the depth of the injury and compare with that shown in Figure 32. Fig. 40. Three weeks after injury, the necrophylactic periderm is not yet confluent with the first exophylactic periderm. Fig. 41. Three weeks after wounding, divisions of the cortical parenchyma are poorly aligned with respect to the plane of injury (arrows). Fig. 42. Shoots collected eight weeks after injury reveal development of the necrophylactic periderm to be the same as that in shoots collected three weeks after injury. Note cortical cell divisions (arrow). Fig. 43. Tissue section from a shoot collected eight weeks after injury and stained with IKI. Note the starch depletion in rays closest to the site of injury (arrows).

Scale bars in Figures 38 to 42 = 50  $\mu$ m.

Scale bar in Figure 43 = 100  $\mu$ m.

All Figures depict transverse sections.

For all Figures: C = cortex, FEP = first exophylactic periderm.





week sooner after injury than shoots wounded in the fall. In addition, suberization of phellem cells occurred almost as soon as the cells were produced by the phellogen, and the necrophylactic periderm may be completed within three weeks of wounding in spring. Necrophylactic periderm development was not complete in shoots wounded in the fall after a period of eight weeks, nor had those cells which developed as derivatives of the necrophylactic periderm become suberized.

With the exception of the present study, seasonal rates of necrophylactic periderm development in immature tissues such as current year shoots, have not been explored. However, these observations are in general agreement with those of Mullick and Jensen (64), who concluded that necrophylactic periderm development in mature bark of several conifer species occurred most quickly in spring, and most slowly in fall and winter. The period of greatest activity of the phellogen has been shown by another investigation (Chapter II) to occur very early in the spring. It seems likely that environmental and physiological conditions during the spring season are most conducive to a rapid wound response by the injured plant.

When inoculum of Nectria coccinea var. faginata was applied to wounds inflicted during the spring, the development of the necrophylactic periderm was rapid enough to limit the spread of the fungus to the necrotic zone. Since suberization of the new phellem occurred within two weeks

of injury, the shoots were protected against pathogen invasion very quickly. Wounds inflicted and inoculated in the fall, however, also remained uninfected at least for a period of up to eight weeks, even though a necrophylactic periderm was incompletely developed. The presence of the fungus did not appear to affect the rate of necrophylactic periderm formation in either the spring or fall trials. This observation is consistent with that of Biggs et al. (7).

Factors other than necrophylactic periderm formation also influence pathogen success (41). Only speculation as to why N. coccinea var. faginata failed to successfully infect fall-inflicted wounds and induce canker formation can now be offered. One possibility is that the fungus itself develops more slowly in the fall, and that the reduction in the rate of necrophylactic periderm formation is of little or no consequence. Another is that if the experiment were continued through the winter, the fungus may have eventually colonized the shoot and induced canker formation. It also should be noted that the tissues inoculated in this experiment are not those normally colonized by this pathogen.

It is plausible that the slower development of the necrophylactic periderm in shoots wounded in the fall may provide a selective advantage to certain pathogens. Butin (20) has shown that an increase in water stress led to a decrease in rate of wound periderm development in Populus spp., and that cankering induced by Cytospora spp. then

occurred. Injuries sustained in the fall by a plant are likely to become successfully infected if the pathogen can arrive at the site, germinate, and invade tissues prior to the development of the necrophylactic periderm. Fungi which grow slowly during the fall, or which do not produce abundant inoculum in this season, may have no advantage in infecting such wounds.

## CHAPTER IV

### CHARACTERISTICS AND DEVELOPMENT OF NECROPHYLACTIC PERIDERMS IN MATURE BARK OF AMERICAN BEECH WITH REGARD TO THE BEECH BARK DISEASE

#### Introduction

Woody plant periderms which arise as the result of mechanical injuries, insect, or pathogen attack have been referred to as wound periderms (17, 84, 85) or necrophylactic periderms (61, 82). As a general rule, such a periderm provides a barrier which effectively limits the spread of pathogenic fungi through the bark tissues and prevents them from reaching the vascular cambium. As a result of their development in response to bark injuries, a certain degree of resistance to pathogen invasion is imparted to the plant (42).

Necrophylactic periderms have been shown or implicated to play a role in tree diseases such as chestnut blight (17), white pine blister rust (84), Nectria canker of hardwoods (36), yellow-laminated root rot of Douglas-fir (61), and the beech bark disease of European beech (5, 18, 19, 33). Regarding the beech bark disease, Braun (18) and Fink and Braun (33) have suggested that the bark damage resulting from the feeding activity of the scale insect Cryptococcus fagisuga allows the bark to dry and crack along the sclerified phloem rays to the vascular cambium. The

presence of the rays themselves may also prevent the wound periderm from forming an uninterrupted barrier to the subsequent invasion of the beech bark by Nectria coccinea.

Although necrophylactic periderm development has been generally accepted as a major defense mechanism in woody plants (42, 61), comparatively few studies have been made of the ontogeny of the process. Some aspects of necrophylactic periderm development have been explored. Temperature (55), growth regulating substances (16), light conditions (11), plant water relations (20, 67), and season of wounding (36, 64) have all been shown to influence necrophylactic periderm development. Differences in these environmental conditions may often account for the success or failure of the plant to defend itself against pathogen ingress. With the exception of a study conducted by Ehrlich (29), the role of the first exophylactic periderm in canker development, and the development of necrophylactic periderms in American beech have not been reported.

The objectives of the present investigation were to (i) describe canker formation on American beech resulting from mechanical injury and inoculation with Nectria coccinea var. faginata during each of the four seasons, (ii) compare necrophylactic periderm development in trees which are susceptible with that in trees apparently resistant to the beech bark disease, and (iii) describe two canker types of the beech bark disease resulting from natural infections on American beech.

## Materials and Methods

### Seasonal Inoculation Study.

Twenty-four American beech, each 5 to 10 cm in diameter, were selected at the Kingman Research Farm in Madbury, New Hampshire. One of four groups of six trees each was selected for treatment in each season. Treatments were initiated in the summer (8/23/79), fall (10/23/79), winter (1/16/80), and spring (4/1/80). Trees for each treatment were harvested for anatomical study two years from the time of treatment initiation.

Five whorls of 12 mm diameter wounds were inflicted on each of five trees in each group. Each whorl consisted of two wounds inoculated with a three week old malt agar culture of a single-ascospore isolate of Nectria coccinea var. faginata, a wound left uninoculated, and an area of the bark inoculated but left unwounded. The wounds were inflicted by the removal of a 12 mm diameter disc of periderm tissue. Wounds were approximately 0.5 mm in depth. All wounds were inflicted the same day in a given season. Wounds to be inoculated were so treated from the top whorl to the bottom whorl after 0, 2 days, 1, 2, and 4 weeks had passed, respectively, since the time of injury. These five trees were designated as the treatment trees.

The remaining tree in each group served as a control for time of inoculation. This tree was wounded, by whorl, at the corresponding treatment time intervals. All wounds were inoculated immediately after being inflicted. This

tree was designated as the control tree.

Trees were inspected regularly for canker development over a period of two years, after which time they were harvested. Representative samples of uninoculated wounds, inoculated wounds, and healthy bark were sectioned to a thickness of 10 to 20  $\mu\text{m}$  on a cryostat set at approximately  $-25^{\circ}\text{C}$ . Sections were either stained with Toluidine Blue O or left unstained, and mounted in glycerol on standard glass microscope slides. Small samples (1 X 1 X 2 mm) of bark tissues were also fixed, dehydrated, infiltrated, and embedded in Spurr's epoxy resin (50). Epoxy embedded tissues were sectioned at 3 to 5  $\mu\text{m}$  with an ultramicrotome equipped with a glass knife. Sections were stained with Toluidine Blue O or left unstained, and examined microscopically.

Development of Necrophylactic Periderm in Susceptible and Apparently Resistant American Beech.

Four pairs of American beech, each pair consisting of one tree susceptible and one tree apparently resistant to the beech bark disease, were selected for study. Two pairs were located at the Hubbard Brook Experimental Forest near West Thorton, New Hampshire, and two were located at the Bartlett Experimental Forest near Bartlett, New Hampshire. Both research areas are administered by the USDA Forest Service.

Trees selected as pairs were a maximum of 2 m apart. Susceptible trees had obvious delimited cankers on which

evidence of fruiting by Nectria coccinea var. faginata could be found. The scale insect Cryptococcus fagisuga was present in moderate to low populations. Apparently resistant trees had no evidence of cankering or fruiting by N. coccinea var. faginata. An occasional individual scale insect was found on some of the apparently resistant trees. All trees were between 19.6 and 30.0 cm in diameter (dbh).

Four whorls of eight injuries per whorl were made between 0.5 and 2 m above the ground on the bole of each tree. Wounds were made by removing a 10 mm diameter disc of tissue approximately 0.5 mm in thickness from the bark. Susceptible trees were wounded where the bark was free of both primary causal agents of the beech bark disease. Wounds were inflicted on 18 and 20 September, 1981.

Wounds of the top two whorls of each tree were left uninoculated; wounds of the bottom two whorls were inoculated with a 10 mm diameter malt extract agar disc from a three week old single-ascospore isolate of N. coccinea var. faginata. Inoculations were made the same day as wounds were inflicted. Bark samples of wounds of both treatments from all trees were collected at approximately bimonthly intervals and analyzed for anatomical changes. The cryostat was used to prepare fresh tissue sections, and the ultramicrotome was used to prepare epoxy-embedded tissues, as described previously.

#### Two Canker Types of the Beech Bark Disease on American Beech.

A large number of American beech growing in natural



forest stands at the Hubbard Brook and Bartlett Experimental Forests were inspected over a three year period. Both research areas are classified as aftermath zones (77) with respect to the beech bark disease. The disease has been present in both areas for approximately thirty years.

Several levels of beech bark disease intensity were observed. Bark samples were collected from healthy trees and from trees exhibiting various degrees of cankering by N. coccinea var. faginata. Sections of bark samples were prepared for microscopic examination as described previously, and anatomical characteristics were related to general canker appearance.

### Results

#### Seasonal Inoculation Study.

Nectria coccinea var. faginata was able to incite cankers on American beech stems when applied to bark tissues from which only the first exophylactic periderm had been removed (Fig. 44). Although many cankers developed, none grew larger than 3.5 cm in length or 2.2 cm in width. The slowest cankers to develop reached a maximum size within a period of approximately 12 months after initiation. No canker enlargement was observed after that time.

Frequency of canker development was influenced by the season in which the trees were wounded and inoculated (Table I). Twenty-two of the fifty wounds inflicted and inoculated in the fall resulted in cankers, compared with 0, 1, and 4 of those inoculated in winter, spring, or

Figures 44 - 47. Necrophylactic periderm development in American beech wounded or wounded and inoculated with Nectria coccinea var. faginata. Fig. 44. Canker development around wounds 18 months after being inoculated. Arrow indicates erumpent sporodochia of the fungus. Fig. 45. Necrophylactic periderm delimiting the canker shown in Figure 44. The wound was inflicted in the fall and inoculated one week later. The resulting canker was harvested two years after the wound was inflicted. Note the shape of the sclerified phloem ray which has been delimited to the necrotic zone, and compare with Figure 25. Fig. 46. Appearance of phloem centripetal to the necrophylactic periderm. Note the long, even radial files of vascular cambium derivatives. Fig. 47. Appearance of the necrophylactic periderm developing behind a wound inflicted in the winter.

Scale bar in Figure 44 = 5 mm.

Scale bars in Figures 45 and 47 = 40  $\mu$ m.

Scale bar in Figure 46 = 20  $\mu$ m.

Figures 45 and 46 depict transverse sections.

Figure 47 depicts a longitudinal section.

For all Figures: NP = necrophylactic periderm,

S = sclerenchyma, SPR = sclerified phloem ray, X = xylem.

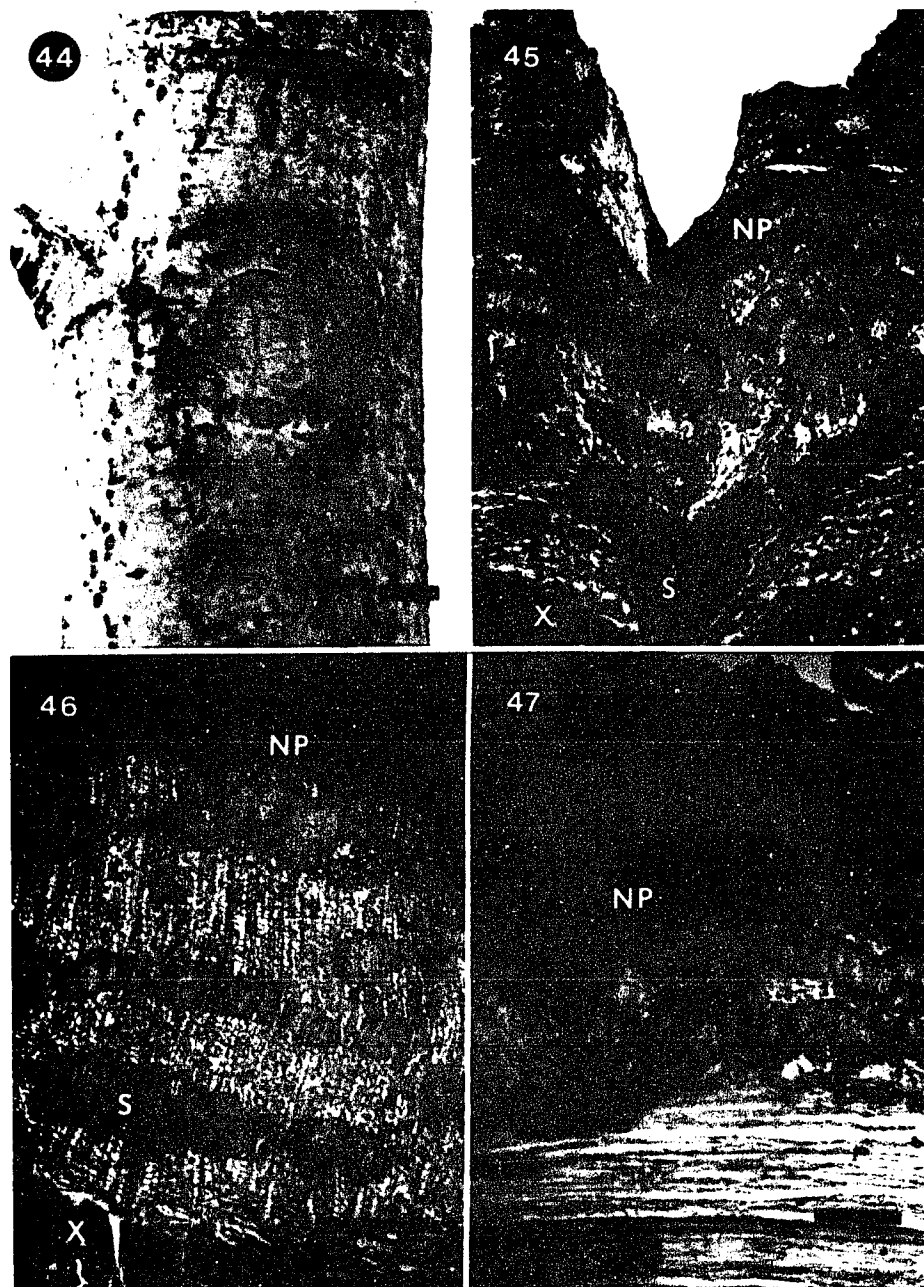


Table I. Effect of season of wounding and inoculation with Nectria coccinea var. faginata on canker development on American beech. Wounds were areas of the bark from which only the first exophylactic periderm had been removed.

| Season of Wounding<br>and Inoculation | Number of Inoculated<br>Wounds Forming Cankers |                            |
|---------------------------------------|--|----------------------------|
|                                       | Treatment Trees <sup>a</sup>                   | Control Trees <sup>b</sup> |
| Spring                                | 1  | 0                          |
| Summer                                | 4  | 1                          |
| Fall                                  | 22   | 7                          |
| Winter                                | 0  | 0                          |

<sup>a</sup>Each value represents the number of wounds forming cankers from a total of 50 inoculated wounds. Wounds were inflicted the same day in a given season but inoculated after various time intervals of up to four weeks.

<sup>b</sup>Each value represents the number of wounds forming cankers from a total of 10 inoculated wounds. Wounds were inflicted at various time intervals but inoculated immediately after being inflicted.

summer, respectively.

The time interval between wounding and inoculation was also found to influence canker development (Table II). The longer the time interval between wounding and inoculation, the fewer cankers developed. No wounds which were left uninoculated resulted in cankers, nor were any cankers formed when inoculum was applied to nonwounded bark.

Canker development on trees used as controls for the time intervals was also affected by season of inoculation. Again, most cankers occurred on wounds inflicted in the fall (Table I). However, canker development became less frequent as the fall season progressed, even when wounds were inoculated immediately after being inflicted. Of the seven fall wounds resulting in cankers, only one of four wounded and inoculated after 15 November resulted in canker formation, compared with six of six wounds inflicted and inoculated prior to 2 November. No cankers developed from wounds inflicted and inoculated during the winter season.

Examination of tissue sections cut from two year old wounds which had resulted in cankers revealed that a well developed necrophylactic periderm had formed. This periderm consisted of a phellem from twenty to twenty-five cell layers in thickness (Fig. 45). A phelloderm could not be distinguished. The necrophylactic periderm was never observed to be interrupted by the sclerified phloem rays. Rather, it always formed a continuous barrier between healthy and necrotic tissue, with the sclerified phloem rays

Table II. Effect of various time intervals between wounding and inoculation of *Nectria coccinea* var. *faginata* on canker development on American beech. Wounds were areas of the bark from which only the first exophylactic periderm had been removed.

| Days Between Wounding<br>and Inoculation | Number of Inoculated<br>Wounds Forming Cankers |                            |
|--|--|----------------------------|
|  | Treatment Trees <sup>a</sup>                   | Control Trees <sup>b</sup> |
| 0  | 10   | 3                          |
| 2  | 7  | 2                          |
| 7  | 3  | 2                          |
| 14                                       | 4  | 1                          |
| 28                                       | 3  | 0                          |

<sup>a</sup>Each value represents the number of wounds forming cankers from a total of 40 inoculated wounds. A total of 10 wounds were inflicted in a given season and inoculated after each given time interval.

<sup>b</sup>Each value represents the number of wounds forming cankers from a total of 8 inoculated wounds. Two wounds were inflicted in a given season and inoculated after each given time interval.

being separated completely from healthy tissues (Fig. 45).

Large groups of sclerenchyma were commonly observed as short bands centripetal to the sclerified phloem rays which had been separated from healthy tissue by the necrophylactic periderm.

Healthy bark tissue was composed of long files of phloem cells (Fig. 46). The cells were uniform in size and shape. Bands of sclerenchyma were a common characteristic of this bark tissue as well (Figs. 46, 47).

#### Development of Necrophylactic Periderm in Susceptible and Apparently Resistant American Beech.

The general appearance of one pair of American beech selected for study is shown in Figure 48. On closer examination (Fig. 49), many small delimited cankers are apparent on the susceptible tree. The bark of some of those cankers show deep splits indicating infestation by the scale insect Xylococcus betulae (Perg.). Even though the trees are touching at the base, one tree of the pair has remained completely free of any cankers and is considered to be apparently resistant to the beech bark disease.

Bark tissues behind wounds of both susceptible and apparently resistant trees eight weeks after injury appeared slightly discolored. Healthy bark tissues appeared light brown or tan, while tissues abutting the wound surface were dark red-brown. Tissues of wounds which had been inoculated appeared even darker, and the extent of the discolored tissues was larger than that of wounds left uninoculated.

Figures 48 - 51. Characteristics of bark of American beech trees susceptible or apparently resistant to the beech bark disease. Fig. 48. Susceptible (right) and apparently resistant (left) American beech at the Hubbard Brook Experimental Forest. Fig. 49. Appearance of cankers (arrows) resulting from natural infection by Nectria coccinea var. faginata. Fig. 50. The appearance of a wound twenty weeks after being inflicted on the bark of a susceptible tree and inoculated with Nectria coccinea var. faginata. No cankering is yet apparent. Fig. 51. Appearance of bark tissue centripetal to the wound inflicted two months earlier. Wounds were inflicted by the removal of only the first exophylactic periderm. This wound was inflicted on an apparently resistant beech, and inoculated with Nectria coccinea var. faginata. Anatomical changes in the bark tissue indicating initiation of a necrophylactic periderm are not apparent.

Scale bars in Figures 48 and 49 = 15 cm.

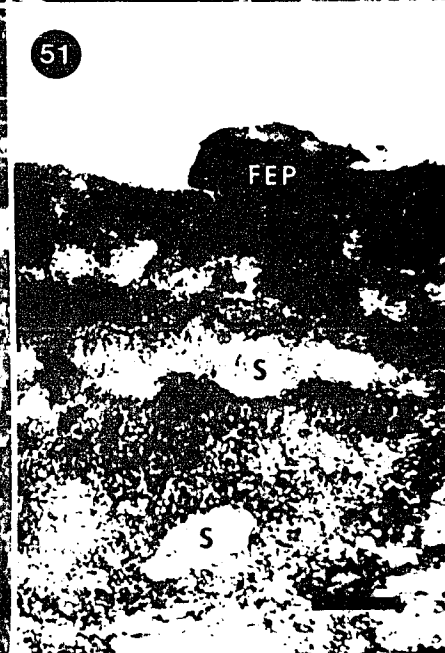
Scale bar in Figure 50 = 5 mm.

Scale bar in Figure 51 = 400  $\mu$ m

Figure 51 depicts a transverse section.

For all Figures: FEP = first exophylactic periderm,  
S = sclerenchyma.





No differences between susceptible and apparently resistant trees in terms of extent or appearance of discolored bark tissues was evident.

The bark surface around wounds 20 weeks after wounds had been inflicted was smooth, with no evidence of cankering (Fig. 50). Fruiting structures of N. coccinea var. faginata were not observed when inoculated wounds were examined. Microscopic examination of tissues from these wounds revealed that the extent of the discolored zone had increased only slightly from that of the eight week old wounds. No anatomical changes were apparent in bark tissues at this time (Fig. 51).

Significant changes in canker appearance and bark anatomy had occurred in tissues collected and examined 38 weeks after injuries had been inflicted. Abundant sporodochia of N. coccinea var. faginata were observed around most inoculated wounds on both susceptible and apparently resistant trees, and a distinct canker margin could be observed (Fig. 52). Anatomical changes in bark tissues behind both inoculated and uninoculated wounds were also evident (Fig. 53). A necrophylactic periderm had been generated and was already confluent with the first exophylactic periderm (Fig. 54). The necrophylactic periderm formed a continuous barrier between healthy and necrotic tissues. It was observed that neither sclerified phloem rays nor large groups of sclerenchyma cells interrupted the necrophylactic periderm (Fig. 55).

Figures 52 to 55. Characteristics of experimentally induced cankers on American beech determined to be either susceptible or apparently resistant to the beech bark disease.

Fig. 52. Canker appearance 38 weeks after the wound was inflicted and inoculated with Nectria coccinea var. faginata. Sporodochia of the fungus are present (arrows). Fig. 53.

Bark sectioned through a wound to show canker extent. The necrotic zone which has developed is delimited from the healthy tissue by a necrophylactic periderm. The necrophylactic periderm can be seen here as a narrow band (arrow) separating the necrotic zone from the healthy region.

Fig. 54. Appearance of the necrophylactic periderm from the canker depicted in Figure 53. The necrophylactic periderm has become confluent (arrow) with the first exophylactic periderm. The necrotic tissues were to the left of the necrophylactic periderm; the healthy tissue is to the right. Fig. 55. Section of tissue from the same canker as that shown in Figure 53. Note how the groups of sclerified cells do not interrupt the continuity of the necrophylactic periderm.

Scale bars in Figures 52 and 53 = 5 mm.

Scale bars in Figures 54 and 55 = 100  $\mu$ m.

Figures 53 to 55 depict transverse sections.

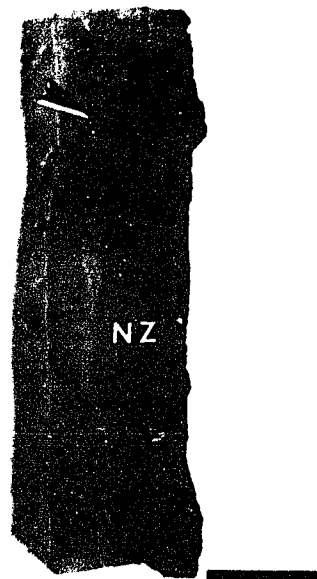
For all Figures: FEP = first exophylactic periderm,

NP = necrophylactic periderm, NZ = necrotic zone,

S = sclerenchyma.



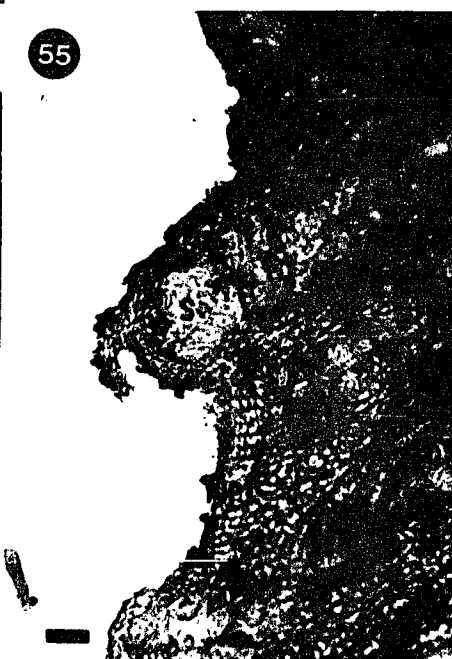
53



54



55



### Two Canker Types of Beech Bark Disease on American Beech.

Two distinct types of bark cankers were evident on trees affected by the beech bark disease. Cankers which were restricted in size and defined in cross section by a distinct necrophylactic periderm were designated as delimited cankers. Cankers not restricted in size usually resulted in large necrotic areas of tree bark, and were often associated with rapid tree mortality. Such cankers were not defined in cross section by a necrophylactic periderm. The necrosis occurred through the bark to the vascular cambium. These cankers were designated as diffuse cankers. It was noted that populations of C. fagisuga were low on trees exhibiting either canker type. However, this does not necessarily reflect past scale populations on those trees.

Characteristics of delimited cankers, the most common type encountered, are shown in Figures 56 to 64. Delimited cankers were small, usually no larger than 1.5 cm in length by 2.5 cm in width (Fig. 56). These cankers usually occurred as individuals, but occasionally two or more had coalesced. Evidence of fruiting by N. coccinea var. faginata could be observed on most delimited cankers (Fig. 57). When observed in cross section, cankers were delimited by a distinct necrophylactic periderm which apparently always formed a continuous protective sheath between the necrotic tissue and the healthy bark (Figs. 58, 59).

Evidence that the necrophylactic periderm of delimited

Figures 56 - 61. Characteristics of naturally induced delimited cankers of the beech bark disease on American beech. Fig. 56. Appearance of typical delimited cankers. Fig. 57. Rupture of the first exophylactic periderm by fruiting structures of Nectria coccinea var. faginata (arrows). Fig. 58. Well defined necrophylactic periderm delimiting necrotic tissue from healthy bark tissue. Note the bands of sclerenchyma developing behind this periderm. Fig. 59. Exclusion of the sclerified phloem rays from the healthy bark tissue by the necrophylactic periderm. Fig. 60. Appearance of the necrophylactic periderm entirely ensheathing the necrotic zone, or canker. Small protrusions (arrows) show where the necrophylactic periderm formed centripetal to groups of sclerenchyma. Fig. 61. Same as in Figure 60, but showing where the necrophylactic periderm formed centripetal to the sclerified phloem rays (arrows).

Scale bar in Figure 56 = 15 mm.

Scale bar in Figure 57 = 2.5 mm.

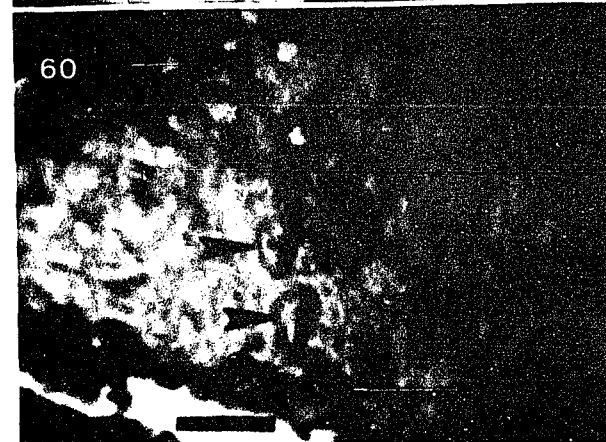
Scale bars in Figures 58 and 60 = 1 mm.

Scale bar in Figure 59 = 300  $\mu$ m.

Scale bar in Figure 61 = 0.5 mm.

Figures 58 and 59 depict transverse sections.

For all Figures: NP = necrophylactic periderm, NZ = necrotic zone, S = sclerenchyma, SPR = sclerified phloem ray.



Figures 62 to 64. Characteristics of the necrophylactic periderm of naturally induced delimited cankers of the beech bark disease on American beech. Fig. 62. Necrophylactic periderm showing sclerified phloem ray excluded from healthy tissue, well developed phellem, and phellogen (arrow). Fig. 63. Detail of the phellem surrounding the sclerified phloem ray shown in Figure 62. Fig. 64. Appearance of sclerenchyma centripetal to the necrophylactic periderm, and the sclerified phloem rays. Compare the general shape of the sclerified phloem ray to that shown in Figures 25 and 45.

Scale bar in Figure 62 = 50  $\mu\text{m}$ .

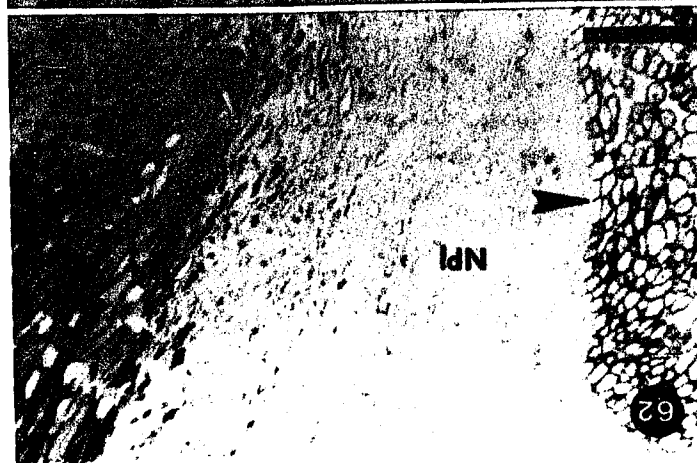
Scale bar in Figure 63 = 10  $\mu\text{m}$ .

Scale bar in Figure 64 = 300  $\mu\text{m}$ .

All Figures depict transverse sections.

For all Figures: NP = necrophylactic periderm, NP1 = necrophylactic periderm phellem, S = sclerenchyma, SPR = sclerified phloem ray.





cankers was continuous was demonstrated by autoclaving bark samples containing cankers for a period of about ten minutes. In all cases the entire canker, consisting of the necrotic bark tissue and the phellem of the necrophylactic periderm, could be easily separated as a single piece from the healthy bark tissue (Figs. 60, 61). The necrophylactic periderm was not interrupted either by large groups of sclerenchyma or by the sclerified phloem rays.

Characteristics of the necrophylactic periderm of delimited cankers included a well developed phellem twenty-five to thirty-five cell layers in thickness, and a single phellogen layer (Figs. 62, 63). A phelloderm was apparently lacking.

Bark tissues centripetal to the necrophylactic periderm appeared similar to those of other healthy bark tissues, with the exception of the arrangement of sclerenchyma. Often, bands of sclerenchyma were arranged so that they formed one to several sheets of tissue immediately centripetal to the necrophylactic periderm (Figs. 58, 59). Other large groups of sclerenchyma were located in alignment with the sclerified phloem rays which had been excluded from the living bark by the necrophylactic periderm (Fig. 64).

Diffuse cankers were observed on only a few trees during the course of this investigation. This canker type is more common in the killing front zone of the disease (77), which is now located in New York and Pennsylvania.

Active diffuse cankers may be recognized by the

relatively large numbers of perithecia of N. coccinea var. faginata on extensive areas of the bark. The bark, however, appears not to have been disrupted in any way. On two occasions, an entire tree bole was found completely covered with the perithecia. The cankers on these trees presumably involved the entire tree stem. In both instances, tree mortality had occurred within a few months after initial observations had been made.

Examination of diffuse cankers in cross section revealed that a necrophylactic periderm had not developed (Figs. 65-68). The bark area between healthy and necrotic tissues was often discolored in a random pattern (Fig. 66). A distinct demarcation between these tissues was not apparent. The exophylactic periderm as well as the parenchyma and other phloem cells were killed before an anatomical response to the advancing pathogen had occurred.

#### Discussion

The seasonal inoculation study has demonstrated that the fungus N. coccinea var. faginata has the capability to grow through healthy bark tissues and incite cankers, thereby acting as a parasite. The fungus lacks the ability to directly penetrate the phellem. Ehrlich (29) also performed experiments with American beech in which only the phellem layer was punctured or removed prior to inoculation with N. coccinea var. faginata. He also reported that infection almost always resulted. However, the influence of season of wounding, and the relationship of infection to

Figures 65 - 68. Characteristics of naturally induced diffuse cankers of the beech bark disease on American beech. Fig. 65. General appearance of bark at the canker margin. Perithecia of Nectria coccinea var. faginata (arrow) are present. Fig. 66. Indistinct boundary between healthy and necrotic tissues. Fig. 67. Extent of necrosis in the first exophylactic periderm (arrow). Note that no apparent anatomical barriers occur in the region between the discolored and healthy areas. Fig. 68. Appearance of phloem tissues taken from the margin of a diffuse canker. Note that there is no strong demarcation between healthy and necrotic tissues.

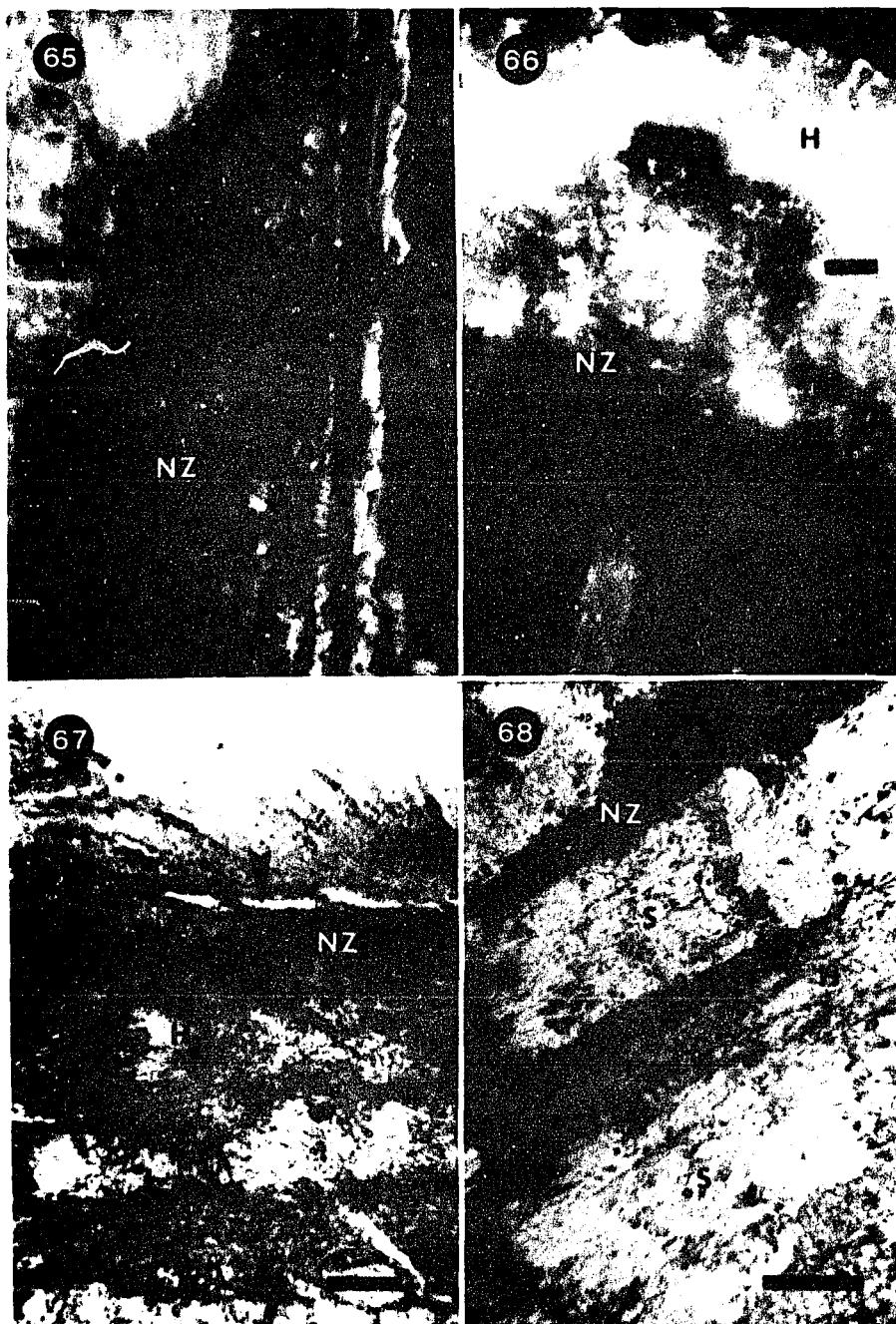
Scale bar in Figure 65 = 1 mm.

Scale bars in Figures 66 and 68 = 100  $\mu$ m.

Scale bar in Figure 67 = 200  $\mu$ m.

All Figures depict transverse sections.

For all Figures: H = healthy tissue, NZ = necrotic zone, S = sclerenchyma.



necrophylactic periderm development was not studied.

The seasonal inoculation study indicates that establishment of N. coccinea var. faginata is most successful when wounds are inflicted and inoculated in the early fall. This is also in agreement with the findings of Grant and Spaulding (36), who studied the effects of season of wounding on canker development resulting from inoculation with another species of Nectria on various species of hardwoods. Injuries made by the feeding activity of C. fagisuga may be more receptive to infection in the early fall, after the eggs have been laid and the adults have died. Ascospore and conidia discharge by N. coccinea var. faginata is also likely to occur during the fall, aided by moist climatic conditions during that time of the year.

For these reasons, it appears likely that the early fall season is critical in terms of beech bark disease development. Wounds have been made by the insect, the fungus is disseminating spores, and bark tissues of the host are slow to respond to injury in terms of necrophylactic periderm development (Chapter III, 64).

Although cankers developed as a result of inoculating wounds, they had become delimited within two growing seasons by a well developed necrophylactic periderm. Perrin (66) and Lonsdale (54) have reported on canker development on European beech resulting from inoculation of N. coccinea on trees stressed by high populations of C. fagisuga. The cankering was less severe when the insect was not present or

was removed. Perrin (66) suggested that it is the continued presence of relatively high populations of C. fagisuga which ultimately results in the inability of the tree to limit canker size. European beech is apparently capable of limiting damage caused by N. coccinea alone (5).

Results of the present study support this concept as well. Inoculated trees were free of C. fagisuga, and were not under the influence of any apparent stress factor. Damage resulting from infection by N. coccinea var. faginata was limited and localized.

Since the fall season was determined as the most important in terms of beech bark disease development, susceptible and apparently resistant pairs of American beech were wounded and inoculated during the fall. A necrophylactic periderm was initiated sometime between 30 and 38 weeks after wounding. This provides N. coccinea var. faginata a long period of time in which to become well established in bark tissues. However, it is unlikely that the fungus is active during this entire period since cankers failed to develop from wounds inoculated in the late fall or winter.

No apparent difference was observed in rate of necrophylactic periderm development between susceptible and apparently resistant trees. It is reasoned that necrophylactic periderm development is controlled to some degree by genetic processes. However, it was not possible to distinguish genetic differences as related to the apparent

susceptibilities of the beech chosen for this experiment.

There was a substantial difference in canker characteristics between inoculated and uninoculated wounds. A clearly discernable canker developed around inoculated wounds, and the necrotic region of these cankers were approximately 1.5 time the diameter of those injuries resulting from uninoculated wounds. Also, sporodochia of N. coccinea var. faginata developed in abundance around inoculated wounds. No differences in characteristics between the necrophylactic periderms of inoculated and uninoculated wounds were apparent.

The characteristics of the delimited cankers from natural infections were very similar to those of cankers induced by mechanical wounding and inoculation (Figs. 45, 59). Sheets of sclerenchyma were arranged in layers centripetal to the necrophylactic periderm, and large groups of sclerenchyma developed in alignment with sclerified phloem rays now included with the necrotic tissue. The necrophylactic periderm had developed as a continuous protective sheath, and was not interrupted by sclerified phloem rays. This observation is not consistent with those reported by Braun (18). It is possible that the European beech used in the latter study does behave differently in terms of necrophylactic periderm formation than does American beech. Specific comparative studies are needed before that question can be resolved.

An hypothesis to explain delimited canker development



is supported by two key anatomical observations. The sclerified phloem rays which were present in the necrotic zone centrifugal to the necrophylactic periderm were always shaped as those in healthy bark (Figs. 59, 62). That is, the ray end closest to the vascular cambium was sharply pointed or tapered. Sclerified phloem rays were always more or less uniform in width except in this region. Secondly, the radial files of cells observed centripetal to bark cankers of the seasonal inoculation study are likely the result of a rapid and uniform development from the vascular cambium.

It seems most likely that the necrophylactic periderm which effectively delimited these cankers, and which formed as a continuous protective sheath originated from cells recently derived from the vascular cambium. Stimulation of the vascular cambium to produce substantially different xylem cells in response to injury has been well explored (56, 78, 87). A different response, one involving the phloem, is also apparently stimulated by certain specific conditions resulting from bark injury.

The fact that a necrophylactic periderm may also be differentiated from existing cells in the bark tissue is also evident from Mullick's investigations (61, 63) and the present study (Figs. 53-55). In such instances, the development of an uninterrupted necrophylactic periderm in American beech may still depend on the ability of the vascular cambium to produce phloem derivatives capable of

differentiating into a new phellogen. However, these events may only occur in the localized areas where sclerified phloem rays are found within a few cells of the vascular cambium (Fig. 53).

In light of the work by Perrin (66) and Lonsdale (54), it may be rewarding to investigate the influence of high populations of C. fagisuga on the vascular cambium. The insect may be capable of preventing the vascular cambium from producing phloem derivatives which are in turn capable of differentiating into a necrophylactic periderm. Bark invasion by N. coccinea var. faginata may then be anatomically unopposed by the host. Once the fungus reaches and kills the vascular cambium, its further progress in the bark tissue is assured.

## CHAPTER V

### EXTRACTABLE PHENOL LEVELS IN BARK OF AMERICAN BEECH IN RELATION TO THE BEECH BARK DISEASE

#### Introduction

Stands of American beech (Fagus grandifolia) in the northeastern United States have been damaged by the beech bark disease since the 1930's. The disease is the result of the action of two primary causal agents; a scale insect Cryptococcus fagisuga, and the fungus Nectria coccinea var. faginata (77). C. fagisuga feeds primarily on the parenchyma cells in the bark and predisposes the bark tissues to subsequent infection by N. coccinea var. faginata and other related species of Nectria (26, 29). Aspects of the etiology and epidemiology of this disease have been described by Ehrlich (29), Shigo (76), and Houston et al. (45).

Apparent differences in susceptibility of beech to the beech bark disease were noted by Shigo (76) in 1964. Trees heavily cankered by the disease were observed growing in close proximity to trees free of both primary causal agents. Tree ages were not noted, but their size indicated that they had been present in the stand for some time prior to the introduction of the disease. Factors which may be involved in determining disease resistance in this host:parasite system have only recently been addressed (43, 93).

The distribution, amounts, and kinds of phenolic compounds present in plant tissues have been shown to significantly influence disease development in many host: parasite systems (42, 52). The role of phenolics with regard to pathogen attack of living trees has been investigated by several researchers (73, 74, 79, 86). Shain (72) described a phenol-enriched "reaction zone" which develops in xylem of species of Pinus and Picea in response to infection by Fomes annosus (Fr.) Cke., and Shortle (79) has described similar zones in response to mechanical injury in species of Acer and Populus. In both instances, the phenol-enriched zone is thought to be a chemical barrier which prevents or retards pathogen movement within the tree. The relationship of bark phenolics to infection of Acer rubrum L. and A. saccharum Marsh. by decay fungi has been discussed by Tattar and Rich (86). Normal levels of bark phenolics were found to be much greater than levels in wood tissues. Hamamelitannin was recently reported to be present in bark of species of Castanea and Quercus susceptible to Endothia parasitica, but absent in bark of those species resistant to chestnut blight, the disease which E. parasitica induces (30).

The present study was undertaken to determine the amount and distribution of total extractable phenols of beech bark with regard to (i) cankers naturally induced by N. coccinea var. faginata, and (ii) experimentally inflicted bark injuries which were either inoculated with the fungus

or left uninoculated, on trees which were either susceptible or apparently resistant to the beech bark disease. Phenol extracts from bark samples of natural cankers and from samples of various experimental treatments were also measured for qualitative differences.

### Materials and Methods

#### Natural Bark Cankers.

Naturally occurring cankers were collected from beech trees at the Hubbard Brook Experimental Forest in West Thorton, New Hampshire. Three delimited cankers from each of three trees were removed by making transverse cuts with a handsaw approximately 0.5 cm above and below each canker to a depth of approximately 1 cm. A chisel was then used to split the intact canker from the tree. Cankers were placed in plastic bags and brought to the laboratory for processing within three hours.

#### Mechanically Inflicted Bark Injuries.

Four pairs of beech trees, each pair consisting of one susceptible and one apparently resistant individual, were selected for experimental treatment. Two pairs were located at the Hubbard Brook Experimental Forest, and two were at the Bartlett Experimental Forest near Bartlett, New Hampshire.

Four whorls with eight injuries per whorl were made between 0.5 and 2 m above the ground on the bole of each tree. Wounds were made by removing a 10 mm diameter disc of tissue approximately 0.5 mm in thickness from the bark. Wounds on susceptible trees were made where the bark was

free of both primary causal agents. Wounds were inflicted on 18 and 20 September, 1981.

Wounds of the top two whorls of each tree were left uninoculated. Wounds on the bottom two whorls were inoculated with a 10 mm diameter malt extract agar disc from a three week old culture of a single-ascospore isolate of N. coccinea var. faginata. Inoculations were made the same day as wounds were inflicted. All wounds were covered with masking tape for a period of one month, after which time the tape was removed.

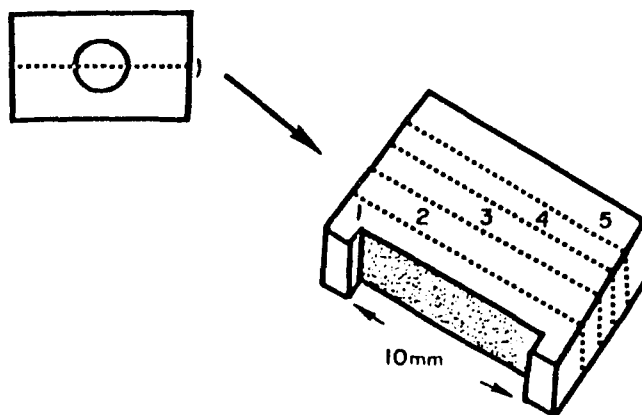
Three inoculated and three uninoculated wounds were collected from each tree at the Hubbard Brook area on 12 March, and from each tree at Bartlett on 22 March, 1982. In addition, three samples of healthy unwounded bark tissue were collected from all trees on 22 March. Samples were collected as described for natural bark cankers. Bark samples of wounds of each treatment from all trees were also collected at bimonthly intervals from time of wounding. These samples were analyzed for anatomical changes, reported in Chapter IV.

#### Processing of Bark Samples.

The collected bark samples were brought to the lab and five sections of approximately 1 X 1 X 10 mm in size were cut from behind the periderm or wound surface to the cambium (Fig. 69). Each section was then dried at 70 C for 24 hours, and stored in glass vials at room temperature until phenol extractions were made. Total phenols of each section were

Figure 69. Sampling procedure for bark phenolics. A 1 mm thick section of bark was cut from the mid-section of each sampled wound, from the bark surface to the vascular cambium. The section was trimmed to a length of approximately 10 mm, the width of the wound. Five 1 mm sections were then cut from the block, with section 1 being the nearest to the wound surface, and section 5 being the nearest to the vascular cambium. Each bark section was then subjected to the phenol extraction procedure.

### Sampling Procedure for Bark Phenolics





extracted in 76% ethyl alcohol at 70 C for one hour, and measured using the Folin-Ciocalteu method (40). Extract absorption was measured using a Bausch and Lomb Spectronic 200 UV spectrophotometer. Extracts from selected bark sections were also analyzed for their absorption spectra.

### Results

#### Natural Bark Cankers.

Extractable phenol levels of tissue sections obtained from natural bark cankers are shown in Table III. Phenols increased in tissues from the necrotic zone with increasing distance from the bark surface from 4.8 to 24.8  $\mu\text{g}/\text{mg}$  dry weight. Tissue sections representing primarily the necrophylactic periderm contained an average total phenol content of 49.1  $\mu\text{g}/\text{mg}$  dry weight. Sections of bark tissue obtained nearest the vascular cambium were found to contain the highest amount of phenols, 71.5  $\mu\text{g}/\text{mg}$  dry weight. These tissues appeared healthy and were apparently free from the influence of N. coccinea var. faginata which was delimited by the necrophylactic periderm.

#### Mechanically Inflicted Bark Injuries.

Phenol levels in tissue sections from unwounded healthy bark of susceptible and apparently resistant trees are shown in Table IV. Sections from bark of susceptible trees were not significantly different in amount of total phenols than corresponding sections from bark of apparently resistant trees. Lowest phenol levels of 36.6 and 41.0  $\mu\text{g}/\text{mg}$  dry weight were found in tissue sections obtained from the mid-

Table III. Total extractable phenols in tissue sections of bark of American beech. Sections were obtained from natural bark cankers induced by Nectria coccinea var. faginata.

| Section<br>Number <sup>a</sup> | Tissue<br>Condition        | Total Phenols<br>( $\mu\text{g}/\text{mg}$ dry wt.) <sup>b</sup> | 95% C.I.<br>(+/-) |
|--------------------------------|----------------------------|--|-------------------|
| 1                              | Necrotic                   | 4.8  | 1.7               |
| 2                              | Necrotic                   | 14.2   | 6.0               |
| 3                              | Necrotic                   | 24.8   | 3.6               |
| 4                              | Necrophylactic<br>Periderm | 49.1   | 4.1               |
| 5                              | Healthy                    | 71.5   | 7.5               |

<sup>a</sup>Section number represents approximate distance in mm from bark surface from which a 1 mm thick tissue section was obtained.

<sup>b</sup>Mean of 9 observations representing 3 cankers from each of 3 trees is represented by each value.

Table IV. Total extractable phenols in tissue sections of healthy bark of American beech determined to be either susceptible or resistant to the beech bark disease.

| Section<br>Number <sup>a</sup> | Total Phenols ( $\mu\text{g}/\text{mg}$ dry wt.) <sup>b</sup> |           | 95% C.I. (+-) |           |
|--------------------------------|---|-----------|---------------|-----------|
|                                | Susceptible   | Resistant | Susceptible   | Resistant |
| 1                              | 48.2  | 46.2      | 3.4           | 4.6       |
| 2                              | 39.5  | 45.7      | 3.1           | 3.9       |
| 3                              | 36.6  | 41.7      | 2.8           | 4.1       |
| 4                              | 45.7  | 41.0      | 5.9           | 4.3       |
| 5                              | 57.0  | 47.3      | 6.4           | 2.9       |

<sup>a</sup>Section number represents approximate distance in mm from wound surface from which a 1 mm thick tissue section was obtained.

<sup>b</sup>Each value represents the mean of 12 observations representing 3 samples from each of four trees.

region of the bark of both susceptible and apparently resistant trees. Similarly, highest phenol levels were found in sections from nearest the vascular cambium.

Data obtained from measurement of total phenol levels in wounded bark tissues of susceptible and apparently resistant trees are shown in Table V. Sections obtained from wounds inflicted on susceptible trees had a lower amount of phenols, on average, than did corresponding sections obtained from wounds of apparently resistant trees. This difference was significant ( $P=0.05$ ) for the three sections of bark representing the mid-region of bark tissues sampled. Phenol content of tissue sections increased as sampling progressed from the wound surface (section 1) to the bark tissue nearest the vascular cambium (section 5) for both susceptible and apparently resistant trees.

A comparison of amount of phenols in wounded bark tissues inoculated with N. coccinea var. faginata with the amount in those left uninoculated is shown in Table VI. Regardless of wound treatment, phenol content of tissue sections increased as sampling progressed from the wound surface to the section nearest the vascular cambium. However, within corresponding sections, a difference between wound treatments is apparent. The first two tissue sections, those nearest the wound surface of inoculated wounds, had a significantly lower ( $P=0.05$ ) amount of phenols than did corresponding sections from uninoculated wounds. The last

Table V. Total extractable phenols in tissue sections from wounded bark of American beech susceptible or apparently resistant to the beech bark disease.<sup>a</sup>

| Section Number <sup>b</sup> | Total Phenols ( $\mu\text{g}/\text{mg}$ dry wt.) <sup>c</sup> |           | 95% C.I. (+-) |           |
|-----------------------------|---|-----------|---------------|-----------|
|                             | Susceptible   | Resistant | Susceptible   | Resistant |
| 1                           | 11.7  | 14.9      | 1.6           | 1.6       |
| 2                           | 14.6  | 28.9      | 1.6           | 3.6       |
| 3                           | 22.2  | 38.0      | 2.1           | 4.1       |
| 4                           | 34.7  | 43.9      | 2.3           | 4.7       |
| 5                           | 52.6  | 54.6      | 4.4           | 4.8       |

<sup>a</sup>Wounds were inflicted by the removal of a 10 mm diameter disc of the first exophylactic periderm to a depth of 0.5 mm. Wounds were either inoculated with Nectria coccinea var. faginata or left uninoculated.

<sup>b</sup>Section number represents approximate distance in mm from the wound surface from which a 1 mm thick tissue section was obtained.

<sup>c</sup>Each value represents the mean of 24 observations representing 3 inoculated and 3 uninoculated wounds from each of four trees.

Table VI. Total extractable phenols in tissue sections from wounds inflicted on bark of American beech, with wounds either inoculated with Nectria coccinea var. faginata or left uninoculated.<sup>a</sup>

| Section<br>Number <sup>b</sup> | Total Phenols<br>( $\mu\text{g}/\text{mg}$ dry wt.) <sup>c</sup> |              | 95% C.I. (+-) |              |
|--------------------------------|--|--------------|---------------|--------------|
|                                | Inoculated   | Uninoculated | Inoculated    | Uninoculated |
| 1                              | 10.0   | 16.6         | 1.5           | 1.4          |
| 2                              | 16.6   | 27.0         | 2.0           | 3.6          |
| 3                              | 26.5   | 33.6         | 3.3           | 3.8          |
| 4                              | 39.0   | 38.7         | 3.6           | 4.0          |
| 5                              | 55.0   | 52.2         | 4.5           | 4.8          |

<sup>a</sup>Wounds were inflicted by the removal of a 10 mm diameter disc of the first exophylactic periderm to a depth of 0.5 mm. Trees were either susceptible or apparently resistant to the beech bark disease.

<sup>b</sup>Section number represents approximate distance from the wound surface from which a 1 mm thick tissue section was obtained.

<sup>c</sup>Each value represents the mean of 24 observations representing 3 wounds from each of 4 susceptible and 4 apparently resistant trees.

two tissue sections, those closest to the vascular cambium of inoculated wounds, did not have a significantly different amount of phenols than did corresponding sections from uninoculated wounds.

The average phenol content of the tissue section nearest the vascular cambium of inoculated wounds was slightly, but not significantly higher than that from the corresponding tissue section of uninoculated wounds. However, when data from the Hubbard Brook tree pairs were analyzed apart from the Bartlett data, a significant difference was found. The phenol content of bark sections nearest the vascular cambium was significantly ( $P=0.05$ ) higher in inoculated than in uninoculated wounds (Table VII). Based on the spectrophotometric scans, qualitative differences in phenols were not found between extracts from tissue sections of various wound treatments and extracts from tissue sections of healthy bark.

### Discussion

The relatively high phenol level in bark tissues of woody plants is generally thought to act as a chemical barrier to the invasion of pathogens (42, 79). Pathogens which incite stem cankers, however, have evolved mechanisms which allow them to either overcome or avoid (46) this barrier.

Nectria coccinea var. faginata can easily be isolated from infected bark tissues in relatively pure culture. Recent studies have indicated that bark tissues stimulate

Table VII. Total extractable phenols in tissue sections from wounds inflicted on bark of American beech at the Hubbard Brook Experimental Forest.<sup>a</sup>

| Section<br>Number <sup>b</sup> | Total Phenols<br>( $\mu\text{g}/\text{mg}$ dry wt.) <sup>c</sup> |              | 95% C.I. (+-) |              |
|--------------------------------|--|--------------|---------------|--------------|
|                                | Inoculated   | Uninoculated | Inoculated    | Uninoculated |
| 1                              | 12.5   | 20.0         | 2.9           | 2.2          |
| 2                              | 19.6   | 30.5         | 3.3           | 6.2          |
| 3                              | 28.4   | 37.2         | 5.2           | 6.1          |
| 4                              | 38.0   | 38.1         | 3.3           | 6.5          |
| 5                              | 57.9   | 45.1         | 5.8           | 4.1          |

<sup>a</sup>Wounds were inflicted by the removal of a 10 mm diameter disc of the first exophylactic periderm to a depth of 0.5 mm. Trees were either susceptible or apparently resistant to the beech bark disease. Wounds were either inoculated with Nectria coccinea var. faginata or left uninoculated.

<sup>b</sup>Section number represents approximate distance in mm from the wound surface from which a 1 mm thick tissue section was obtained.

<sup>c</sup>Each value represents the mean of 12 observations representing 3 samples from each of 2 susceptible and 2 apparently resistant trees.



the fungus to produce perithecia (24). Nectria coccinea var. faginata does not require direct access to the vascular cambium or xylem in order to produce asexual or sexual stages, as evidenced by its fruiting on delimited cankers.

The results of the present study, when considered with observations mentioned above, indicate that N. coccinea var. faginata is quite tolerant of the phenolic environment of American beech bark. This is significant in that fungi capable of breaking down chemical barriers may persist for long periods of time in a comparatively inactive state. Shortle (79) has suggested that phenols act as growth regulating substances of decay fungi. Phenols in bark tissues may act in a similar manner to bark parasites such as N. coccinea var. faginata.

Once chemical and physical barriers to infection are breached, the pathogenic fungi are already in an advantageous position to continue the disease process. Physical barriers, such as the necrophylactic periderm, may be ruptured by infestations of Xylococculus betulae or by other means. Chemical barriers may be slowly broken down by the action of N. coccinea var. faginata itself.

The sampling procedure for total phenols used in this study have led to the discovery that bark phenols are not evenly distributed throughout tissues of either healthy or wound-altered bark of American beech. Healthy bark tissues of American beech which are nearby the vascular cambium or cork cambium have slightly higher phenol levels than tissues

in the mid-region of the bark. Perhaps this is a reflection of the number of living cells capable of producing secondary metabolic products in these regions. The mid-region of American beech bark was observed to contain more sclerenchymatic tissue than regions closer to the vascular cambium or cork cambium. Sclerenchymatic tissue is likely metabolically inactive, and therefore low in phenols.

The uneven distribution of phenols in bark is even more pronounced in tissues around inoculated and uninoculated wounds. Wounding by removal of the exophylactic periderm resulted in a decrease of bark phenols in tissues centripetal to the wound surface. The rate of decrease appears to be accelerated when N. coccinea var. faginata is present. Some non-decay fungi have been shown to be capable of altering these substances (80).

Further, there is at least some evidence (Table VII) to indicate that the presence of N. coccinea var. faginata on the wound surface may cause the host response of increased phenol production in bark tissues at some distance from the infection site. Although this relationship could not be shown to be statistically significant for all four tree pairs, data from two of these pairs indicated that this effect was significant. All of the data represent phenol content of tissues at one point in time; six months after injury. Changes in phenol levels in advance of fungus invasion has been suggested as a defense mechanism of living

xylem tissues (W. C. Shortle, personal communication). It is possible that a high phenol level in bark tissues is a dynamic barrier, "retreating" as the fungus advances but, by its presence, slowing that advance. Such an hypothesis may be tested by conducting a time course study.

Wound-altered bark tissues of susceptible trees which were inoculated with *N. coccinea* var. *faginata* had less phenols than those of apparently resistant trees (Table V). It is suggested that the susceptible trees may be lower in vigor due to the fact that numerous natural cankers were already present. This could account for the apparently poor ability of the tree to maintain high phenol levels in the event of pathogen invasion. However, a second possibility is that susceptible trees inherently produce phenols more slowly than resistant trees. As phenols are lost either through wounding, pathogen utilization, or both, their replacement occurs at a slower rate. Testing of either hypothesis will have to wait until such time when it becomes possible to distinguish susceptible and apparently resistant trees in areas free of the beech bark disease.

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## APPENDIX

## ELECTRICAL MEASUREMENTS OF PHELLOGEN

The phellogen of beech is superficial and relatively uniform in thickness and depth from the bark surface. On older trees, the phellogen is separated from the vascular cambium by several mm of secondary phloem and "expansion" tissues. Measurements of the electrical resistivity (ER) of each cambium is feasible due to these unique properties of beech bark. The objective of this investigation was to determine if the individual contributions of the phellogen and the vascular cambium to total "vigor ER", as measured by the Shigometer<sup>®</sup>, could be quantified. If this were possible, it may be a useful technique with which to study phellogen activity throughout a season or when influenced by controlled environmental treatments.

Vigor ER readings were taken with the Shigometer using the 10mm long needle probes (10 mm actually able to penetrate the tissue). Needle probe bases were insulated with a 0.25 mm thick plastic disc.

ER readings were taken by inserting the probes their entire length into the tree. Two measurements were taken on each tree at each sampling date. Measurements were taken approximately 1 m above the ground. These readings represented vigor ER with periderm. After these readings were obtained, the first exophylactic periderm near each measurement site (within 5 mm) on the bark was removed by scraping with a sharp knife. A wound of about 10 X 20 mm was thus made. A second set of ER measurements was then taken. These represented vigor ER without periderm.

Ten trees in each of six diameter classes (5-cm classes from 1 to 30 cm) were measured as described above every two weeks from March 10 to October 29, 1981. Results are shown in the following table.

Electrical resistance measurements taken on  
sixty American beech trees between March 10,  
1981 and October 29, 1981 at the  
Kingman Research Farm

| <u>Date</u> | <u>Electrical Resistance Reading</u> |                                     | <u>LSD<sup>b</sup></u> |
|-------------|--------------------------------------|-------------------------------------|------------------------|
|             | <u>With Periderm</u>                 | <u>Without Periderm<sup>a</sup></u> |                        |
| 3/10        | 50.98                                | 51.23                               | 3.80                   |
| 3/24        | 53.30                                | 51.10                               | 4.86                   |
| 4/7         | 32.50                                | 29.50                               | 2.62                   |
| 4/21        | 29.70                                | 25.20                               | 2.25                   |
| 5/5         | 19.40                                | 17.30                               | 1.73                   |
| 5/19        | 25.40                                | 22.10                               | 2.70                   |
| 6/2         | 18.20                                | 15.50                               | 1.70                   |
| 6/11        | 15.70                                | 13.90                               | 1.44                   |
| 6/25        | 16.20                                | 15.90                               | 1.96                   |
| 7/9         | 12.42                                | 12.33                               | 1.57                   |
| 7/23        | 14.07                                | 14.18                               | 1.75                   |
| 8/6         | 15.30                                | 15.20                               | 1.75                   |
| 8/20        | 20.92                                | 21.26                               | 2.49                   |
| 9/3         | 22.57                                | 20.64                               | 2.07                   |
| 9/17        | 25.73                                | 25.27                               | 2.04                   |
| 9/30        | 37.90                                | 39.28                               | 3.60                   |
| 10/14       | 37.30                                | 35.40                               | 3.06                   |
| 10/29       | 35.69                                | 36.34                               | 2.91                   |

<sup>a</sup>Each value is the average of 120 readings; two readings per tree on each of sixty trees.

<sup>b</sup>Least Significant Difference with  $P = 0.05$ .